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Bridging the omega-3 gap: The disparity between actual and target intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in children

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BRIDGING THE OMEGA-3 GAP: THE DISPARITY BETWEEN ACTUAL AND TARGET INTAKES OF EICOSAPENTAENOIC ACID (EPA) AND DOCOSAHEXAENOIC ACID (DHA) IN CHILDREN

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This thesis is dedicated to my husband who has taught me to push perceived limitations, exceed extraordinary expectations, and remember that anything is possible. Thank you for patiently reminding me that wisdom cannot be learned simply by reading, but must be gained with an open mind and by learning from experiences of my own and others. Thank you for all that you have endured and all that you have provided to give me the support that I need and become the successful person I am today. I am ever so grateful.

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ABSTRACT

Huss, Lyndsey R. M.S., Purdue University, May 2014. Bridging the Omega-3 Gap: The Disparity Between Actual and Target Intakes of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) in Children. Major Professor: Sibylle Kranz.

Omega-3 (n-3) PUFAs have long and flexible structures that prevent the membranes of human cells from becoming too rigid for nutrients and wastes appropriately permeate the cell membrane. The most abundant n-3 long chain polyunsaturated fatty acid in the cell membranes of the brain and retina is docosahexaenoic acid (DHA), which has a major structural and functional role in cognitive and visual development and function. However, Western diets are high in n-6 PUFAs, which compete with n-3 PUFAs for the same set of metabolic enzymes and cause the phospholipid bilayer of our cell membranes to become rigid and brittle, decreasing the permeability of the cell membranes and preventing nutrients and wastes to enter and exit the cell.

Many Americans focus on the intake of alpha-linolenic acid (ALA) which is an essential fatty acid that can be converted to eicosapentaenoic acid (EPA, important for cardiovascular health), and to DHA. However, less than 1% of ALA is actually converted to DHA. The conversion is highly inefficient in humans, which led to the classification of DHA as a “conditionally-” or “physiologically-” essential fatty acid.

Although ALA is an essential fatty acid and part of a healthy diet, if the goal is to increase DHA, foods with a high DHA density must be consumed.

Good sources of EPA and DHA are foods from marine sources such as fish oil, krill oil, algal oil, and animal based foods such as fish and shellfish, but also poultry, eggs, and pork. In addition, many new food products have been developed that are fortified with EPA and/or DHA. Although fish, EPA and DHA have been promoted by the food market, the data on children's consumption of fish and intake of EPA and DHA is limited. For that reason, the Institute of Medicine (IOM) has not been able to set Dietary Reference Intakes for EPA and DHA despite the accumulation of research stressing the importance of these two n-3 long chain PUFAs for human health.

To improve children's diet quality for optimal growth and development and the prevention of chronic disease, we conducted two studies. One was focused on analyzing nationally representative data; the other was designed to test the feasibility of children accepting and consuming oily fish in a childcare setting. The objective of our first study was to provide evidence for 2-18 year old children's fish and shellfish consumption patterns and their EPA and DHA intake. The aims of this study were to estimate proportion of children consuming fish, to identify major dietary sources of EPA and DHA, and to estimate intake of EPA and DHA. Dietary and socioeconomic data from children 2-18 years old from the National Health and Nutrition Examination Surveys 2003-2010 were used to determine the EPA and DHA density of the foods consumed as well as the intake on a) the nutrient level (EPA, DHA), b) the food level (salmon, chicken, eggs) and c) the Food Frequency Questionnaire level (food consumption in past 30 days). Overall, results showed that those foods with the highest EPA and DHA density were the least consumed by children. Children who had at least some EPA and DHA intake received these two nutrients from foods with low EPA and DHA density, such as ice cream. However, half the American children consumed fish or shellfish, which is the food group that contributes the greatest amount to the intake of EPA and DHA. Salmon was a prevalent source of EPA and DHA in all age groups.

The objective of the second study was to increase children's intake of DHA by providing salmon during lunch for children attending childcare. The aims of this study were to introduce salmon without significantly affecting 2-5 year old children's energy intake per meal and to examine children's acceptance of salmon dishes. Forty-five two-to-five year old children were exposed to four chicken and four comparable salmon dishes over the course of a two-month period. The plate-waste method was used to estimate consumption, and a 3-point Likert scale was used for children to rate their acceptance of each chicken and salmon dish. Statistical analysis included the comparison between total energy and DHA intake of meals and the comparison of children's ratings of the liking of each food. Our results showed that children had comparable intake and acceptance of the salmon macaroni and cheese and salmon salad wraps, two dishes that were more familiar to the children. EPA and DHA intake was significantly increased in the sample. Overall, the majority of children consume larger quantities of foods with low EPA and DHA density. However, it is very possible to increase children's EPA and DHA intake by adding foods with higher EPA and DHA density to children's diets.

CHAPTER 1. INTRODUCTION

1.1 Aims

Current epidemiological and experimental research indicates that consumption of fish high in n-3 polyunsaturated fatty acids (PUFAs), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), could play a role in cerebral function and visual acuity in humans. To contribute to the body of knowledge on children's fish consumption, two studies were conducted for this thesis project. The aims of the first study were 1) to estimate proportion of children consuming fish, 2) to identify major dietary sources of EPA and DHA, and 3) to estimate intake of EPA and DHA. The aims of the second study were 1) to introduce salmon without significantly affecting 2-5 year old children's energy intake per meal and 2) to examine acceptance of salmon dishes.

1.2 Organization

This thesis project has been written in the non-traditional format. In this format, chapter two is a detailed review of literature including the research hypothesis that was tested. In addition, mechanisms for how docosahexaenoic acid may be incorporated into child-friendly foods are discussed along with modes to market fish to young children. Chapter 3 includes the manuscript that will be submitted for publication to the *Journal of Nutrition*, reporting that fish consumption of 2-18 year old children from the large, nationally representative data set, NHANES. Chapter 4 includes the manuscript that has been published in the journal of *Food and Nutrition Science*, providing the methods, results, and discussion on a study investigating the feasibility of developing child-friendly fish dishes to increase young children's fish consumption and acceptance. Finally, a brief conclusion follows based on study results, and future directions are addressed.

CHAPTER 2. LITERATURE REVIEW

2.1 Dietary Fatty Acids

Some dietary fatty acids must be ingested by humans due to the body's lack of enzymes to endogenously synthesize these fatty acids, although they are required for biological processes. There are two essential fatty acids that cannot be synthesized by humans: linoleic acid (LA), an n-6 polyunsaturated fatty acid (PUFA), and α-linolenic acid (ALA), an n-3 PUFA. Arachidonic acid (AA) is an n-6 PUFA that can be synthesized from LA and the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be synthesized from ALA. However, synthesis of EPA and DHA from ALA may be insufficient under certain conditions.

2.1.1 The Importance of the n-6: n-3 Fatty Acid Ratio

The characteristic Western diet tends to be much higher in n-6 PUFAs than n-3 PUFAs with a ratio up to 16.7:1(1). However, previous research suggests that humans evolved on a diet with the ratio of n-6: n-3 PUFAs being 1:1 (2). In addition, the conversion of LA and ALA to docosapentaenoic acid (DPA) and DHA, respectively, depends on the ratio of n-6: n-3 PUFAs (3). Lower ratios of n-6: n-3 PUFAs increase endogenous conversion of ALA to EPA and DHA (4). Although there is a large body of literature supporting the notion that the conversion of ALA is influenced by the ratio of n-6: n-3 PUFAs (1-3, 5-9), Goyens et al. proposed that independent of the ratio, the conversion of ALA to EPA and DHA was <0.1% of dietary ALA and amounts of LA and ALA in the diet determined ALA conversion to EPA and DHA (10). To attain a lower n-6: n-3 PUFA ratio, there would need to be a 4-fold increase in fish consumption in the US (4) unless alternative strategies (i.e. food fortification) are implemented to increase n-3 PUFA intake in the US population.

2.1.2 Metabolism and Bioavailability

Fatty acids must be hydrolyzed by pancreatic enzymes from dietary fats such as triglycerides, phospholipids, and cholesterol prior to absorption (11). For the incorporation of fatty acids into micelles, bile salts must be present in the small intestine. Under normal conditions, fat absorption is 85-95% efficient throughout the small intestine. Through a series of desaturation and elongation reactions, humans can synthesize longer n-6 and n-3 PUFAs from the essential fatty acids LA and ALA, respectively (3). In the synthesis of longer PUFAs, such as AA and EPA, LA and ALA compete for the same desaturation and elongation enzymes. ALA is preferred over LA as the substrate of the delta-6 desaturase enzyme; however, excess dietary intake of LA in comparison to ALA results in greater formation of AA than EPA (3). However, if the ratio of LA to ALA is near equal, the body will have the capacity to convert ALA primarily to EPA and DHA (12, 13).

2.1.3 Conversion of ALA to EPA and DHA

The conversion of ALA to DHA is very inefficient. The majority of ALA is oxidized rather than being converted to EPA and DHA (14). By using compartment models, previous studies have estimated the conversion of ALA to DHA to be <1% (15, 16). By measuring peak or area under the curve plasma contents of the labeled fatty acids, <8% of ALA is converted into EPA and <4% is converted to DHA in men (12). However, in women, <21% of ALA is converted to EPA and <9% is converted to DHA (13). This higher conversion rate is due to the effects of estrogen (17). Since conversion of ALA to EPA and DHA is relatively inefficient, this suggests that ALA exerts potential beneficial effects for chronic diseases through conversion to EPA and DHA (18), thus, EPA and DHA may be "conditionally essential" (19, 20). In addition, limited storage of n-3 PUFAs in adipose tissue suggests that a continuous dietary supply of n-3 PUFAs is needed (14). However, Arterburn et al. studied dose response of n-3 PUFAs in humans, and concluded that the best way to increase specific n-3 PUFAs in plasma, tissues, or human milk is to supplement the diet with the fatty acid of interest (14).

2.2 Importance of DHA for Children

Both n-6 PUFAs and n-3 PUFAs are important structural components of cell membranes by affecting fluidity, flexibility, permeability, and the activity of membrane-bound enzymes when incorporated into phospholipids throughout the lifespan (21). DHA plays important roles in nervous system function and vision as the n-3 PUFA is incorporated into the cell membranes of postsynaptic neurons and the cell membranes of the retina, especially during childhood in states of rapid growth and development.

During childhood, n-3 PUFA intake is associated with healthy lifestyle indicators such as higher physical activity levels, discussing "good nutrition" with family members (parents and siblings), and eating school lunch (versus not eating lunch) (22).

2.2.1 Brain Development

In the phospholipids of the gray matter of the brain, DHA and AA are both found in very high proportions (23, 24). This suggests that DHA and AA are important for the development and function of the central nervous system, specifically neurogenesis, neurotransmission, and protection against oxidative stress (21, 25-27). In animal studies, researchers have shown that depletion of DHA in the brain can result in learning deficits. Changes in DHA content of cell membranes of neurons may alter the function of ion channels, membrane-associated receptors, and/or the availability of neurotransmitters (26).

There is extensive research on the benefits of DHA for children's learning and memory. In 2011, Boucher et al. (28) provided the first neurophysiologic and neurobehavioral evidence of long-term beneficial effects of n-3 PUFAs in utero on memory function in school-age children. Children with a mean age of 11-years-old who had higher umbilical cord plasma concentrations of DHA had significantly greater recognition memory than children with lower prenatal DHA intakes. In 2009, Dalton et al. (29) conducted a trial on 7-9 year old children studying the effect of an experimental, n-3 PUFA rich, fish-flour bread spread on cognition of children and discovered that the children randomly assigned to consume the spread had a significant improvement in verbal learning ability and memory. In addition, Richardson et al. (30) found that DHA supplementation improves reading and behavior in healthy but underperforming 7-9 year old children from mainstream schools. In 2012, de Groot et al. (31) observed 700 Dutch adolescents (ages 12-18 years) and concluded that the students with higher fish intake had more advanced vocabulary and higher end term grades. Thus, not only does prenatal DHA intake provide long-term beneficial effects, but DHA intake during childhood also provides immediate beneficial effects on brain development and function.

2.2.2 Visual Acuity

In retinal cell membranes, DHA is found at very high concentrations. Even when n-3 PUFA intake is low, the retina preserves and reutilizes DHA (32). According to animal studies, DHA is required for normal retinal development and function. During development of the retina, there is a crucial period when inadequate DHA will result in permanent abnormalities in function of the retina (23, 32). DHA plays an important role in the regeneration of the visual pigment rhodopsin that is crucial in the visual transduction system that converts visible light to visual images in the brain (33). In 2007, Birch et al. (34) evaluated visual and cognitive outcomes of infant formula supplemented with DHA and AA at 4 years of age. The children who did not have infant formula supplemented with DHA and AA had poorer visual acuity than the children who had been breast-fed or fed an infant formula supplemented with DHA and AA. DHA intake during gestation has beneficial effects for early development and long-term function of the visual parvocellular pathway in children ages 10-13 years old (35).

2.2.3 Motor Function

Motor function is known to be associated with cognition, and has been studied to determine the relationship between motor function at 7 years of age and DHA and AA values both in umbilical plasma and between venous plasma at 7 years of age (36). As measured with the Maastricht's Motor Test, results showed a significant contribution of cognitive performance, age, and gender to the motor function (36). There was a positive relation between umbilical plasma DHA concentration and movement quality outcomes, but not movement quantity (36). In addition, there was a negative, albeit not significant, relation between umbilical plasma AA concentration and movement quality outcomes. Developmental problems, such as attention-deficit hyperactivity disorder (ADHD), and learning problems may be predicted by movement quality (36). These findings align with the concept that PUFA status is associated with developmental and learning disorders such as ADHD (37). Although umbilical plasma levels had a significant influence on quality of motor function, venous plasma sampled at 7 years of age had no significant association with motor function (36). This suggests that PUFA availability and dietary intake early in life may be more important for motor function at 7 years of age than PUFA intake at 7 years of age.

In another study, Chung et al. (22) analyzed the 2000-2004 Hawaii Nutrition Education Needs Assessment Survey data in a sample of 666 children (ages 9-10 years old). The researchers found that fish consumption and n-3 PUFA intake was positively associated with physical activity. This analysis provided evidence that n-3 PUFAs are essential nutrients that are associated with healthy lifestyle indicators, such as physical activity.

2.3 Deficiency of Dietary Fatty Acids

2.3.1 Dietary Fatty Acid Deficiency Symptoms

Impaired growth, dry and scaly skin, polydipsia, polyuria, increased susceptibility to infection, and poor wound healing are among symptoms of a deficiency in essential fatty acids in infants and children (37-40). Behavioral, sensory, and neurologic dysfunction in rats and monkeys have been associated with n-3 fatty acid deficiency (41-43). n-3, n-6, and n-9 PUFAs compete for the same desaturation enzymes. n-3 PUFAs are preferred by the desaturation enzymes; however when dietary intake of n-3 PUFAs is low, n-6 PUFAs are preferred by the desaturation enzymes. When n-3 and n-6 fatty acid statuses are low, n-9 PUFAs are synthesized specifically to Mead acid. Therefore, Mead acid is considered a marker of essential fatty acid deficiency (44). Another indicator of dietary fatty acid deficiency is a ratio of Mead acid: AA >0.2 (40, 45).

2.3.2 n-3 PUFA Deficiency

n-3 PUFA deficiency can lead to visual problems and sensory neuropathy, which can be reversed and resolved through administration of a lipid emulsion containing ALA (46). Dietary Reference Intakes for DHA have not been established although it is well known that plasma DHA concentrations decrease when n-3 PUFA intake is inadequate. In the past, researchers have demonstrated significant impairment on learning and memory in rodents with n-3 PUFA deficiency (47, 48). Due to the significance of this impairment, trials to determine the impact of n-3 PUFA status on cognitive development and decline in humans are underway.

2.4 Food Sources and Dietary Intake of Fish, EPA and DHA in Children

With the increasing popularity and public awareness of the importance of incorporating DHA into the habitual diet, numerous alternative sources of n-3 PUFAs have been developed over the years to supplement the naturally occurring sources of DHA. Furthermore, dietary intake recommendations of seafood, specifically fish, and DHA have been undergoing constant review and debate. To determine dietary intake recommendations, current intake by the American population needs to be assessed to determine consumption levels. Individuals need to be aware of the differences of total lipid composition, EPA content, and DHA content between wild-caught and farmed-raised Alaskan salmon. Consuming salmon is a feasible venue to increase the dietary intake of fish, EPA, and DHA in children.

2.4.1 Food Sources of n-3 PUFAs

DHA is only found in animal tissue lipids, with oily fish being the best dietary sources (49). It is not present in plant sources (such as vegetable fats and oils, grains, nuts, and seeds), although those may provide other n-3 PUFAs (49). Because humans lack specific desaturases, they are unable to form n-3 or n-6 PUFAs de novo and must obtain these PUFAs from their diet (23). DHA is the major n-3 PUFA esterified in the glycerophospholipids that form the structural matrix of brain grey matter and retinal membranes (50, 51). Therefore, DHA accumulation in the brain and retina as well as in other organs depends on the amount and types of n-3 PUFAs consumed in the diet. In addition, dietary intake of n-6 PUFAs is important, as n-6 PUFAs interact and compete with n-3 PUFAs in the fatty acid metabolic pathway (5-8, 52-54).

Once the importance of EPA and DHA was established, numerous alternative sources of n-3 PUFAs were developed. The only natural sources of DHA are marine food sources (e.g. fish), their products (e.g. fish oil, krill oil, and algal oil), and animal-derived foods (e.g. poultry, eggs, beef, and pork) (55-57). Food companies have developed venues to incorporate DHA and other n-3 PUFAs into foods such as breads and pastas, dairy products, eggs, poultry meat, processed meats, salad dressings, margarine, mayonnaise, peanut butter, pizza, nutrition bars, cereal, cereal (granola) bars, yogurt and juices (56-60). By adding DHA to non-marine food sources, a cost-effective venue (in comparison to purchasing oily fish) has been created to increase consumers' intake of EPA and DHA through already commonly consumed foods (61). Although foods fortified with DHA may be more affordable, they are not cost-effective in that they are low in DHA density compared to oily fish and other marine sources.

The fortification of DHA is based on marine sources such as fish or algal oils. There are many issues that should be considered. For instance, the bioavailability of n-3 PUFAs in foods fortified with DHA is understudied. Furthermore, foods with added DHA usually contain a total of 12-50 mg EPA and DHA. The mean DHA amount in one serving of these processed foods is 32 mg, which is equivalent to less than a teaspoon of a salmon fillet. With product claims such as "good source of DHA," parents may be misled into thinking that the consumption of this food may be sufficient to meet their child's need. While the population seems to have accepted these alternative sources of DHA and other n-3 PUFAs, skepticism remains in the field of nutrition.

2.4.2 Toxicological Issues Associated with Fish Consumption

Due to the focus of the thesis at hand, this topic will only be briefly addressed but not discussed in great detail. Although there are many health benefits to consuming fish, real and perceived toxicological issues are associated with fish consumption (62-64). Unfortunately, there remains confusion among consumers and health professionals. Foran et al. (65) analyzed the benefits and risks of consuming farmed and wild salmon to conclude that the relatively low risk of exposure to contaminants in farmed and wild salmon is offset by the health benefits of n-3 PUFA intake. Even though there are many types of fish that are moderate-to-high in environmental pollutants, such as mercury and polychlorinated biphenyls (PCBs), and should not be consumed, there is a category of fish that is low in mercury and PCBs but high in n-3 PUFAs (EPA and DHA) and can be consumed by up to 12 ounces per week, even by children (66). These include the species of anchovy, herring, whitefish, trout, and salmon (66, 67).

2.4.3 Intake Recommendations

According to the IOM report (68), it is recommended that 2-5 year old children consume two age-appropriate servings (1-to 2-ounces) of seafood per week. However, the quantity requirements of total fat and PUFAs have not yet been adequately established (69). Currently, recommended DHA intake levels are based on values calculated on a per kilogram (kg) body weight basis. According to Koletzko et al (70), EPA and DHA intake recommendations using the estimation based on body weight may result in suboptimal DHA amounts in 2-12 year old children, as fast-growing bodies have higher DHA needs relative to their body weight. In adulthood, DHA needs are lower compared to the time from birth to age 25 years old. Although the IOM, international authorities, and expert groups have supplied and/or incorporated dietary recommendations for fish consumption and EPA and DHA intake (63, 71-73), to date, the scientific basis for dietary reference intakes (DRIs) for EPA and DHA is lacking (74). There is a dire need to establish DRIs for EPA and DHA; however, more research is needed to determine EPA and DHA intakes required for optimal growth and development and for prevention of chronic disease and deficiency. It is important to establish age-appropriate guidelines for children whose nervous system function, visual acuity, and motor function are most influenced by EPA and DHA intakes.

2.4.4 Fish Consumption and Dietary Intake of EPA and DHA in Children

Ethnicity, age, and geographical region are factors that help determine the predicted consumption of fish by an individual (75). Based on the estimated intake of DHA in Americans, especially American children, the development of venues to increase fish consumption by offering child-friendly fish dishes is a critical public health concern. In a previous study (76), when pasta was served to 23 two-to-five year old children, food intake was 81% greater than when fish was served. This is the case even for immigrants from Asia who would be expected to have a high fish intake (77). When assessing the dietary patterns of Korean adolescents, fish dishes such as kimchi, fish cake soup, and fish cutlets comprised the majority of meals, whereas Korean-American adolescents, who had acculturated, consumed a more typical American diet of milk, soda, hamburgers, etc. (77).

Despite the benefits of DHA in the diet, Western diets are low in n-3 PUFAs, especially ALA found in plant oils and DHA found in fish (49). In the US, the mean intake of n-3 PUFAs is 0.7% of energy (4), and the median intake of EPA and DHA in adults is 0.05% of dietary energy (4, 72). Even if small amounts of fish are consumed, n-3 PUFA status is significantly improved compared to individuals who do not consume any fish (78). Tran et al. (79) found that the top three fish most frequently consumed, over a 30-day period from the National Health and Nutrition Examination Survey (NHANES) 1999-2006 data, are tuna, salmon, and breaded fish.

According to the NHANES 2003-2008 data, the primary sources of EPA and DHA in the diets of young children (12-60 months of age) in the US are fish, shellfish, and poultry, but 46.3% of young children did not eat fish in the previous month (80). Fish and shellfish contributed 46.3% of DHA to young children's dietary intake, with 24.5% coming from poultry and poultry dishes, 19.6% from eggs and egg dishes, and 2.2% from pasta, rice and other grain dishes (80). For each age group (12-24, 25-36, 37-48, and 49-60 months) and each race and ethnic group (Non-Hispanic white or black and Mexican American), white fish was the most frequently consumed type of fish, followed by shellfish, and then oily fish (80). There are vast discrepancies when comparing estimated EPA, DHA, and total n-3 PUFA intake of US children to other countries (Australia, Belgium, Canada, and China). In the US, young children have lower EPA (6 mg/d) and DHA intake (20 mg/d) than the other countries (17-60 mg/d EPA and 23-96 mg/d DHA) (80, 81). Moreover, US children have a higher mean intake of total n-6 PUFAs (8.6 g/d) when compared to the other countries (2.14-7.60 g/d) (80).

Due to the low conversion rate of ALA to DHA, even an individual meeting the estimated intake recommendations for n-3 PUFA intake may consume less than optimal amounts of DHA, if the majority of the n-3 PUFAs are from plants. Consequently, this may become an issue for the developing brain. Western diets low in n-3 PUFAs and high in n-6 PUFAs may contribute to poor brain development and function (49). Furthermore, fish consumption has been found to be highly correlated to quality of life in the general population, and is associated with healthy lifestyle indicators such as higher physical activity levels (22, 82). Adding oily fish to children's diets may increase DHA intake and may aid in the prevention of poor brain development. Dalton et al. (29) showed that when seven-to-nine year old children's diets were supplemented with a fish-flour spread rich in n-3 PUFAs, verbal learning ability and memory were improved. Thus, effective ways to encourage habitual consumption of a diet high in DHA are warranted. The degree to which current intake practices of U.S. children are reflecting suboptimal brain function is not known.

2.4.5 Wild versus Farmed Atlantic Salmon

Depending on location, season, water temperature, age, sex, and diet, wild salmon vary in total fat, EPA content, and DHA content (83). For farmed salmon, the environment is controlled, yet the EPA and DHA content of the feed vary (83). After comparing the lipid composition in farmed and wild salmon, Hamilton et al. (84) found that farmed salmon had greater levels of total lipid (mean 16.6%) than wild salmon (mean 6.4%). Even though the farmed salmon had higher n-3 and n-6 levels in comparison to the wild salmon, the n-3 to n-6 ratio was about 10 in wild salmon and 3-4 in farmed salmon (84). On the other hand, Blanchet et al. (85) found lipid and n-3 PUFA content of farmed and wild Atlantic salmon to be similar. However, total n-3 and n-6 PUFA levels were significantly higher in farmed Atlantic salmon compared to the wild variety. Although there is natural EPA and DHA variability in wild salmon and variability of feed for farmed salmon (83), both types are still considered good sources of healthy n-3 PUFAs (84) and the best sources of DHA (49).

2.4.6 Recipe Development

To increase consumers' intake of oily fish, recipes can be developed that incorporate fish, fish protein, and fish oils. Cooking methods can affect the nutrient content in a food. Baking does not decrease n-3 PUFA content, thus, baked salmon is an acceptable preparation method to high n-3 PUFA intake (86). In 2006, Serna-Saldivar et al. demonstrated that it was feasible to produce breads fortified with n-3 PUFAs, containing 25-50 mg of DHA per slice (32 g) (87). In 2011, Shaviklo et al. fortified starchy snacks with fish and fish proteins that were widely accepted by 6-16 year old children in two communities (Iceland and Iran) (88). Canned, smoked, or fresh salmon can be used to develop recipes (89). Developing foods that are child-friendly and incorporate fish can increase children's intake of DHA.

2.5 Research Goals, Objectives, and Aims

The overall goal of this research project was to improve children's diet quality for optimal growth and development and for the prevention of chronic disease. The objective of the first study was to provide evidence for children's fish and shellfish consumption patterns and the EPA and DHA intake from a representative sample of US children 2-18 years old in the NHANES 2003-2010. To address children's developmental stages, analysis was conducted using three mutually exclusive age groups (2-5, 6-11, 12-18 years old). The following specific aims outline our approach:

1. Aim 1 was to estimate proportion of children consuming fish. Hypothesis: Children's actual fish and seafood consumption will be rare and not include large proportions of fish high in n-3 PUFAs. Children's reported diets would reflect suboptimal fish consumption (defined by the recommended fish intake in the DGA).
2. Aim 2 was to identify major dietary sources of EPA and DHA. Hypothesis: Major sources of consumption will be foods low in EPA and DHA, which are highly consumed by children. The types of fish most widely consumed by children will not be good sources of EPA and DHA, and the associations between fish intake and EPA and DHA levels will be low. Other foods, not naturally high in EPA and DHA (fortified milk, orange juice, eggs, etc.) will contribute to children's overall EPA and DHA intake level.
3. Aim 3 was to estimate intake of EPA and DHA. Hypothesis: Intake of EPA and DHA will not meet recommended intakes as in the current dietary recommendations set forth by the Food and Agriculture Organization and World Health Organization.

All analyses controlled for children's total dietary intake (serving of fish per 1,000 kcal consumed) to correct for the effect of large food consumption on fish density in the diet. Thus allowing the comparison between food nutrient composition and reference daily values on a standard, per 1,000 kcal basis (90), resulting in a nutrient density ratio that is independent of serving size (91). This is of importance as serving sizes vary among 2-18 year old children.

The second study was designed to explore two modes of increasing children's intake of DHA by providing salmon during lunch for 2-5 year old children attending childcare – by incorporating it in well-accepted dishes and by offering novel dishes. The specific aims of the second research project were 1) to introduce salmon without significantly affecting 2-5 year old children's energy intake per meal but significantly increasing DHA intake and 2) to examine acceptance of salmon dishes. We hypothesized that familiar salmon dishes will not significantly affect energy intake, children will accept the salmon dishes that are more familiar, and offering foods with canned or cooked salmon to preschoolers during lunch at the childcare center will lead to children's consumption of fish at least once a week.

CHAPTER 3. FOOD SOURCES OF EPA AND DHA IN THE DIETS OF AMERICAN CHILDREN, NHANES 2003-2010

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3.1. Abstract

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are n-3 long chain polyunsaturated fatty acids (PUFAs) important for cardiovascular health and neuronal and visual development and function, respectively. However, Western diets are low in n-3 PUFAs (EPA and DHA) and high in n-6 PUFAs. The current study provides evidence based on nationally representative data of children's EPA and DHA intake as well as fish and shellfish consumption patterns. This study used United States National Health and Nutrition Examination Survey data from four waves, 2003-2010. Participants included 13,441 children ages 2-18 years old stratified into three distinct age groups (2-5, 6-11, and 12-18 years old). Less than one-half of the 2-18 year old population reportedly consumed fish (49.0%) and shellfish (35.9%), which are the foods with the highest EPA and DHA densities. The foods that the majority of children reportedly consumed that provided EPA and DHA were ice cream (3.6 mg/day), salty snacks (2.4 mg/day), and eggs (39.0 mg/day). Although children reportedly consumed foods with lower EPA and DHA content but in greater quantities, the "fish and shellfish" food group ranked first in

mean intake of EPA and DHA per day by all children. Overall, results indicate suboptimal consumption of fish and shellfish and intake of EPA and DHA, which suggests need for improvement in the diets of American children. Further research is needed to determine how to best increase children's intake of EPA and DHA to provide health benefits.

3.2. Introduction

The human body has the ability to elongate and desaturate alpha-linolenic acid (ALA) to the long chain n-3 polyunsaturated fatty acids (n-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (92-94). However, Western diets are low in n-3 PUFAs, specifically ALA found in plant oils and EPA and DHA found in oily fish (1, 49, 95). In the United States (US), the mean intake of n-3 PUFAs is 0.7% of total energy consumed (4), and intake in adults is very low with a median intake of EPA and DHA only consisting of 0.05% of total dietary energy (4, 72). Individuals with low fish consumption still have greater n-3 PUFA statuses than those of individuals who do not consume any fish (78).

Western diets low in n-3 PUFAs and high in n-6 PUFAs contribute to poor brain development and function (49, 96). It is uncertain if the rate of DHA synthesis in the human body is sufficient to support optimal brain and retinal development, and therefore it is ideal to obtain these PUFAs from the diet (23). DHA is the major n-3 PUFA esterified in the glycerophospholipids, through the action of acyl-CoA synthases and acyl-CoA: lysophospholipid acyltransferases (97), that forms the structural matrix of brain grey matter and retinal membranes (50, 51). Therefore, DHA accumulation in the brain and retina, as well as in other organs, depends on the amount and types of n-3 PUFAs consumed in the diet. In addition, dietary intake of n-6 PUFAs plays a role as n-6 PUFAs interact and compete with n-3 PUFAs in the fatty acid metabolic pathway (6-8, 52-54, 98). By adding fatty fish to children's diets, EPA and DHA intake would increase and aid in the prevention of poor brain development (96).

To assess fish consumption in Americans over a 30-day period, a supplemental survey focusing on habitual fish intake was added to the National Health and Nutrition Examination Survey (NHANES) 1999-2006. Tran et al. (79) found that the three most frequently consumed fish are tuna, salmon, and breaded fish. According to NHANES 2003-2008 dietary intake data, the food groups that contributed the greatest amount of DHA to the diets of American children were fish and shellfish (46.3%), poultry and poultry dishes (24.5%), eggs and egg dishes (19.6%), and pasta, rice and other grain dishes (2.2%) (80). For all age groups, (12-24 months, 25-36 months, 37-48 months, and 49-60 months) and each races and ethnic groups (Non-Hispanic white or black and Mexican American), the type of fish children most frequently consumed was white fish, followed by shellfish, and then oily fish. Thus, even young children who consume fish are likely not consuming enough oily fish that provides the conditionally essential fatty acids, EPA and DHA.

Fish consumption is likely affected by cultural intake habits. There are large differences in the estimated EPA, DHA, and total n-3 PUFA intake consumed by US children compared to children in other countries (Australia, Belgium, Canada, and China). In the US, young children have the lowest reported EPA (6 mg/d) and DHA (20 mg/d) intakes compared to children in Australia, Belgium, Canada, and China (mean ranges of 17-60 mg/d EPA and 23-96 mg/d DHA) (80, 81). At the same time, US children have a greater mean intake of total n-6 PUFAs (8.6 g/d) compared to Australia, Canada, and China (6.20-7.60 g/d, 7.40-7.74 g/d, and 2.14-2.32 g/d, respectively) (80).

Multiple factors influence the n-3 PUFA content of natural sources of EPA and DHA, even within each food type (i.e. location, season, water temperature, age, sex, and diet) (83). EPA and DHA are found in animal tissue lipids, with oily fish being the best dietary source (49). Other natural sources of EPA and DHA are products of marine sources (e.g. fish oil, krill oil, and algal oil) and animal-based foods (e.g. poultry, eggs, beef, and pork) (55-57). DHA is not naturally present in plant sources (such as vegetable fats and oils, grains, nuts, and seeds), although these may provide other n-3 PUFAs such as ALA (49).

In addition to the natural food sources of EPA and DHA, numerous alternative sources of n-3 PUFAs are available in the US food market. Food production companies have incorporated n-3 PUFAs into breads and pastas, milk, eggs, processed meats, salad dressings, margarine, mayonnaise, peanut butter, pizza, nutrition bars, cereal, yogurt and juices (56-60). Incorporating n-3 PUFAs into commonly consumed foods that do not naturally contain n-3 PUFAs provides a cost-effective, sustainable venue to increase the consumers'' n-3 PUFA consumption, specifically EPA and DHA (61, 99). Although the ALA, EPA, and/or DHA used in the fortification of foods is based on primarily plant (ALA) or marine sources (EPA and DHA), the bioavailability of n-3 PUFAs from fortified foods is currently understudied. Although n-3 PUFAs might be present in the fortified foods, the body's ability to absorb and utilize those fatty acids is a critical step in determining their usefulness. Sanguansri et al. (100) recently showed that different food products fortified with microencapsulated fish oil and fish oil gelatin capsules had equal bioavailability, however, the authors were unable to distinguish if the n-3 PUFA degradation resulted from digestive enzymes produced by the body or by bacteria. Consumer education is needed to help Americans differentiate the sources of n-3 PUFAs and their health benefits.

The 2010 Dietary Guidelines for Americans provide evidence-based nutrition information and advice for people ages two and older. Currently, they recommend that Americans consume seafood at least twice a week with "an intake of 8 or more ounces per week (less for young children)" that will provide a mean of 250 mg per day of EPA and DHA (101). Furthermore, the Joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) Expert Consultation on Fats and Fatty Acids in Human Nutrition have provided adequate intake levels of EPA and DHA for children and adults. The adequate intake levels are as follows: 100-150 mg for 2-4 year olds (age adjusted for chronic disease prevention), 150-200 mg for 4-6 year olds (bridged from an infant value of 10 mg/kg), 200-250 mg for 6-10 year olds (to the adult value assigned at age 10 years), and 250-2000 mg for adults (69). Although national and international public health organizations, professional organizations, and expert committees have made dietary recommendations for fish consumption or EPA and DHA intake (63, 71-73), to date, the Institute of Medicine has not established dietary reference intakes (DRIs) for EPA and DHA (74). To develop and release public health nutrition policy, EPA and DHA target intake levels, such as would be expressed in DRIs for EPA and DHA, must be established. Currently, substantial evidence indicates that these dietary fatty acids have many health benefits (74), play important roles in heart health (102), brain (23-28, 30, 31), and eye development (32-35).

The overall goal of this project was to provide new evidence on the food sources of EPA and DHA in the diets of American children to advance public health policy in determining realistic methods to increase children's intake of EPA and DHA. The objective of this research was to provide evidence, based on nationally representative data, of children's EPA and DHA intake as well as fish and shellfish consumption patterns. The aims of this study were to estimate the proportion of children consuming fish, to identify major dietary sources of EPA and DHA, and to estimate intake of EPA and DHA. To further the understanding of the types of fish and shellfish consumed by American children, the amount and type of fish and shellfish reportedly eaten, especially those with high n-3 PUFA content will be described. The hypotheses tested were that the majority of children do not consume fish, the major dietary sources of EPA and DHA are not widely consumed but sources low in EPA and DHA are more widely consumed, and intake of EPA and DHA does not meet current recommendations set by the FAO and WHO.

3.3 Methods

From children (n=13,441) ages 2-18 years in the NHANES 2003-2010, we analyzed dietary and socioeconomic data including sex, race/ethnicity, and poverty income ratio (PIR) (Table 1). PIR is the ratio of family income to the appropriate poverty threshold (103). Ratios below 1.00 indicate that the family income is below the poverty threshold whereas a ratio of 1.00 or greater indicates income above the poverty threshold. For example, a PIR of 1.25 indicates that family income is 125% of the appropriate poverty threshold. Households with a PIR <1.3 are eligible for the United States Department of Agriculture (USDA) Supplemental Nutrition Assistance Program (SNAP); households with a PIR ≤1.85 PIR are eligible for participation in the USDA Women, Infants, and Children (WIC) Program; households with a PIR of 1.86-3.49 are defined as medium income; households with a PIR of 3.50-5.00 are defined as high income with all values >5.00 truncated to five (104). The PIR is used routinely to express the available income of households, accounting for the number of individuals living in the household. Since the NHANES are publicly available de-identified data, this study was deemed "exempt" by the Institutional Review Board for Human Research of Purdue University.

3.3.1 Nutritional Variables

We used the 24-hour dietary recalls to rank-order (highest to lowest) the foods reportedly consumed by 2-18 year old children by EPA and DHA density (mg/g of food). We identified and described three levels of EPA and DHA consumers (in tertiles of EPA and DHA density). We analyzed the data using three food intake levels: the nutrient intake of EPA and DHA (nutrient-level data), intake of foods per day with food codes indicating fish and shellfish (food-level data), and the responses to the habitual fish intake tool of NHANES to assess usual consumption of seafood, the fish and shellfish food frequency questionnaire (FFQ) used to estimate children's fish and shellfish consumption in the past 30 days (FFQ-level data). To code the nutrient and food-level data intakes, we used the USDA's Food and Nutrient Database for Dietary Studies (FNDDS), 5.0 (2012) (105), and we stratified the analyses by the three age groups (2-5, 6-11, and 12-18 year old children).

3.3.2 Statistical Analysis

We corrected all analyses for survey design and weighted all analyses (using the standard one-day dietary weight) to maintain the nationally representative character of the data. We conducted all analyses in SAS V9.3 (SAS Institute Inc., Cary, NC, USA). We used Proc SurveyFreq in SAS to estimate the percentage of the population in each race/ethnic, PIR, and sex category and to estimate the proportion of children who ate fish or shellfish in the past 30 days as reported in the FFQ. To determine the foods with the highest EPA and DHA density, we selected all individual food items that children in each age group reported and then ranked them in descending order of EPA and DHA density. By calculating the total intake of EPA and DHA for each food item reported by each child, we examined EPA and DHA intake. Then we estimated the mean intake of each individual food item and food group for each age group. We ranked foods containing EPA and DHA in descending order of the milligram weight of EPA and DHA intake per day and in descending order of the number of children reporting the food item. In addition, we ranked food groups in descending order of the milligram weight of EPA and DHA consumed per day. Data are reported as mean ± standard error or as percentages.

3.4 Results

This study included a nationally representative sample of children whose sociodemographic characteristics reflect the demographic profiles of the American population with approximately 50.7% of the children male, 60.2% non-Hispanic white, and 32.0% from a household with a PIR < 1.30 (Table 1). Tertiles of total dietary EPA and DHA density in foods reportedly consumed by 2-18 year old reporters were <5, 5-21, and >21 mg/100g of food for the lowest, medium and highest tertiles. The foods with the highest EPA and DHA density (mg/100g food) reportedly consumed by any children ages 2-18 years old are reported (Table 2A). Although all foods reportedly consumed by the population were included in the analysis, the food sources with the highest EPA and DHA density were from the fish and shellfish food group, with the exception of cooked brains. The foods with the greatest densities of EPA and DHA were sturgeon roe, baked/broiled mackerel, and skinless, boneless, water-packed sardines. Of foods consumed by 2-5 year old children, skinless, boneless, water-packed sardines, pickled herring, and steamed/poached salmon were the most EPA and DHA dense foods (Table 2B). Dried squid, canned salmon, and baked/broiled salmon were the foods consumed by 6-11 year olds that had the highest EPA and DHA densities. As for 12-18 year olds, sturgeon roe, baked/broiled mackerel, and skinless, boneless, water-packed sardines were the foods reportedly consumed having the highest EPA and DHA densities. Out of all of these foods, only one child reportedly consumed each food on the day interviewers conducted the 24-hour dietary recall, except three children consumed the sardines, three children consumed the pickled herring, and 34 children consumed the salmon, baked or broiled. However, we must keep in mind that this is out of a total sample population of 13,441 children.

Since 19 of the 20 foods high in EPA and DHA reportedly consumed by 2-18 year old children were derived from the fish and shellfish food groups, we calculated the proportion of children who reportedly ate fish or shellfish in the previous 30 days. Overall, the majority of 2-18 year old children (82.5%) do have some EPA and DHA intake (any intake greater than zero) from their habitual diet with similar percentages across age groups (83.5%, 84.8%, and 80.1% for 2-5, 6-11, and 12-18 year olds, respectively). However, only 35.9% reportedly consumed shellfish and 49% reportedly consumed fish. The percentage of children consuming shellfish in the past 30 days was 31.6%, 35.9%, and 38.6% for 2-5, 6-11, and 12-18 year old children, respectively. As for consumption of fish in the past 30 days, the percentage of children was 54.5%, 52.3%, and 42.8% for 2-5, 6-11, and 12-18 year old children, respectively.

The greatest mean EPA and DHA intake by foods reportedly consumed by children ages 2-18 years old are reported (Table 3). Overall, 2-18 year old children have a mean EPA and DHA intake of 48 ± 0.002 mg/d. This value varies upon age group, with 37 ± 0.002 mg/d, 45 ± 0.003 mg/d, and 58 ± 0.003 mg/d of EPA and DHA for children ages 2-5, 6-11, and 12-18 years old, respectively. However, when children that do not have any EPA and DHA intake were excluded, the mean daily intake of EPA and DHA increased to 59 ± 0.002 mg/d for 2-18 year old children, 44 ± 0.003 mg/d for 2-5 year old children, 53 ± 0.004 mg/d for 6-11 year old children, and 72 ± 0.004 mg/d for 12-18 year old children. Furthermore, when we included only those children who ate fish and shellfish during the past 30 days, mean EPA and DHA intake further increased, with 64 ± 0.003 mg/d for 2-18 year olds (62.0% consumed seafood), 49 ± 0.004 mg/d for 2-5 year olds (63.4% consumed seafood), 61 ± 0.006 mg/d for 6-11 year olds (63.7% consumed seafood), and 77 ± 0.004 mg/d for 12-18 year olds (59.8% consumed seafood). The foods that provided the most EPA and DHA to those individual children's diets were skinless, boneless, water-packed sardines, cooked salmon, and floured/breaded, fried carp according to the 24-hour dietary recall. Furthermore, we analyzed all the foods that contained EPA and DHA to determine which foods children reported most frequently

(Table 4). Of these foods, ice cream, salty snacks, and eggs ranked as the top three EPA and DHA-containing foods that children reportedly consumed.

In addition, we identified the food sources that contributed the greatest to dietary intake of EPA and DHA among all children, with the number of children reportedly consuming foods from these food groups ranging from 63 to 2248 children (Table 5A). Fish and shellfish; meat, poultry, fish with nonmeat items (mixed dishes); and frozen and shelf-stable plate meals, soups and gravies were the most prominent contributors of EPA and DHA intake. Fish and shellfish; frozen and shelf-stable plate meals, soups and gravies; and egg mixtures were the three most prominent food groups for 2-5 year old children (Table 5B). For 6-11 year old children, the food groups that contributed the greatest were fish and shellfish; meat, poultry, fish with nonmeat items; and egg mixtures. As for 12-18 year old children, the food groups were fish and shellfish; meat, poultry, fish with nonmeat items; and frozen and shelf-stable plate meals, soups and gravies.

3.5 Discussion

Based on a nationally representative sample of US children, we found that among children ages 2-18 years old, the majority of children (82.5%) have some EPA and DHA intake according to their 24-hour dietary recall. Sources of EPA and DHA in the diets of children ages 2-18 years of age include fish and shellfish. However, when it comes to the frequency of 2-18 year old children reporting that they consumed fish or seafood during the past 30 days, only 35.9% consumed shellfish and 49% consumed fish. Of the 2-5 year old children, 48.8% have a family PIR <1.85, indicating eligibility for participation in the WIC program. When analyzing the different age groups, there were increases with age for shellfish consumption but decreases with age for fish consumption.

Fish and shellfish remained the greatest contributing food group to overall EPA and DHA intake in 2-18 year old children although the majority of children did not ever habitually consume fish or shellfish. There is a need to establish a DRI for EPA and DHA as there is substantial evidence that these dietary fatty acids have many health benefits; however, current intake patterns are not well understood, and more research is needed to determine age-appropriate EPA and DHA intake requirements for optimal growth, development, and prevention of chronic diseases. The 2010 Dietary Guidelines for Americans currently recommend consuming seafood at least twice a week as a guideline with a total of 8 ounces per week and “less” for small children (101).

The foods with the greatest EPA and DHA density (mg/100g food) that were consumed by any 2-18 year old child were roe, mackerel, and sardines (Table 2A). Sardines, dried squid, mussels, cooked brains, swordfish, and oysters (Table 2B) were among the reported foods that have the highest EPA and DHA densities. Although these EPA and DHA-rich foods were reportedly consumed by 2-18 year old children, these foods are not commonly consumed by the majority of children. Three children consumed the sardines, one child consumed the dried squid, three children consumed the mussels, one child consumed the cooked brains, two children consumed the swordfish, and one child consumed the oysters. The majority of children do not consume these foods most likely due to barriers such as lack of food access (i.e. ability to obtain or retrieve food), lack of availability (i.e. the quantity and quality of food that is provided by caretakers) (106), and the social context in which the food is encountered such as role models not eating seafood and/or habitual consumption i.e. the children are not used to the odor or flavor of these food sources (107). With this in mind, we must also assess from what food sources the majority of children are obtaining their EPA and DHA intake.

The weighted mean amount of EPA and DHA consumed per day by 2-18 year olds was 48 mg/day. For 2-5, 6-11, and 12-18 year olds, the weighted means for EPA and DHA were 37 mg, 45 mg, and 58 mg, respectively. Considering the Joint FAO and WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition Adequate Intake levels of EPA and DHA for children are 100-150 mg for 2-4 year olds, 150-200 mg for 4-6 year olds, 200-250 mg for 6-10 year olds, and 250-2000 mg for people 10 years and older (69), the current mean amount of EPA and DHA consumed per day is well below these recommendations. The foods that contributed the most EPA and DHA according to foods reportedly consumed per day for 2-18 year olds were sardines, salmon, and carp (Table 3); however, very few children reported eating these foods. The proportion of children who were consuming these foods is small in comparison to the sample population of 13,441 children ages 2-18 years old. This demonstrates the need for public health initiatives to focus on the promotion of safe food sources with high EPA and DHA densities to increase children's acceptance and consumption.

Since the majority of children were not consuming the foods high in EPA and DHA, we analyzed what the majority of children reportedly consumed that contained EPA and DHA (Table 4). Ice cream (n=989), salty snacks (n=581), and egg omelet or scrambled egg (n=537) were the foods with EPA and DHA most commonly consumed by 2-18 year olds. However, these foods provide amounts of EPA and DHA that are well below recommendations (i.e. ice cream, 3.6 mg; salty snacks, 2.4 mg; egg omelet or scrambled egg, 39.0 mg). Although these foods were consumed by more children and contain EPA and DHA, they do not significantly contribute to adequate consumption levels. In the 50 foods contributing the most EPA and DHA that children consumed, tuna salad was the only fish/shellfish-based food and ranked in the thirty-eighth place. The tuna salad was reportedly consumed by 127 children and provided a mean of 167.6 mg of EPA and DHA/day.

From Tables 5A and 5B, we see that fish and shellfish was the greatest contributing food group to dietary intake of EPA and DHA among 2-18 year olds and within each age group. Although fish and shellfish provided the most EPA and DHA on the individual food level and on the food group level as a whole, too few children regularly consumed these foods. Thus, an important priority is increasing the frequency and amounts of these foods consumed by children. Specifically for 2-5 year old children, we have shown that young children will accept oily fish when incorporated into familiar foods such as macaroni-and-cheese and wraps (108). By doing so, we were able to provide a plausible approach to increase fish consumption and significantly increase DHA intake.

The present study had several strengths and limitations. The strengths include, but are not limited to, the large sample size and representation of the US population, the use of validated instruments to estimate EPA and DHA intake, and the pooling of four waves of the NHANES (2003-2004, 2005-2006, 2007-2008, and 2009-2010). One limitation was the analyses were based on one 24-hour dietary recall and a fish and shellfish FFQ. The 24-hour dietary recall is one of the most commonly used dietary intake estimation methods, which has been validated for adults and children in the US

(109-112). A review of dietary data collection methods conducted by Morgan et al. demonstrated that 24-hour dietary recalls provide comparable data to other methods and provide reduced effort and cost (113). However, it does not provide a good estimate of usual intake as usual intake varies considerably from day to day. In addition, fish and shellfish are rarely consumed foods and therefore the FFQ is justified in comparison to the 24-hour dietary recall method. The FFQ was used to estimate children's fish and shellfish consumption in the past 30 days to retrieve a more accurate estimation of how many children consumed seafood within a 30 day time period.

3.6 Conclusion

This study provides detailed information on dietary intake of EPA and DHA among 2-18 year old children. Results indicate that fish and shellfish provide the highest EPA and DHA density, but these are not commonly consumed foods and the majority of children are not consuming any fish or shellfish with 49.0% of 2-18 year olds reportedly consuming fish and 35.9% reportedly consuming shellfish. Increasing consumption of fish and shellfish and of EPA and DHA fortified foods are two initiatives that could be engaged to improve intakes of EPA and DHA in the diets of US children. Future research on this topic should investigate the barriers (e.g. fish consumption advice, availability of and accessibility to oily fish, preparation ability by parents, parental eating habits, and cost) as to why children are not eating fish. Furthermore, public health efforts to increase fish and shellfish intake must be supported. Currently, WIC covers the cost of canned pink salmon and canned white tuna for fully breastfeeding mothers. However, only children that are being fully breastfed or children that have younger siblings being fully breastfed benefit from this WIC food package that is specific to fully breastfeeding mothers. Revising the food package to allow all WIC-eligible mothers to purchase canned fish for their children and themselves will help support the public health effort to increase children's fish consumption. The present study serves as a starting point for the current status of children's fish and shellfish consumption, the food sources that are providing the most EPA and DHA from the foods children reportedly consume, and which food sources most children are reportedly consuming that contain EPA and DHA. With this information, future research can be planned and implemented to determine how to best increase children's intake of EPA and DHA to provide health benefits.

Table 3.1 Population characteristics of children 2-18 year old with non-adjusted diet records, NHANES 2003-2010 [1]

	Total Weighted percent (n=13441)[2]	2-5 years old Weighted percent (n=3380)[3]	6-11 years old Weighted percent (n=4185)[4]	12-18 years old Weighted percent (n=5876)[5]
Male	50.7	51.8	50.4	50.4
Ethnicity				
Mexican American	13.4	15.6	13.9	11.8
Non-Hispanic White	60.2	56.0	59.4	63.0
Non-Hispanic Black	14.4	14.2	14.5	14.4
Other Race	12.1	14.2	12.2	10.8
Income				
<1.30 PIR	32.0	36.8	32.1	29.2
1.30-1.84 PIR	11.1	12.0	12.1	9.8
1.85-3.49 PIR	25.5	25.1	24.8	26.2
3.50-5.00 PIR	31.4	26.1	31.0	34.7

[1]Data are presented as percentages
[2]Family PIR frequency missing=793
[3]Family PIR frequency missing = 206
[4]Family PIR frequency missing = 220
[5]Family PIR frequency missing = 367

Table 3.2A Top 20 foods with highest EPA + DHA density reportedly consumed by children ages 2-18 years old[1]

Food Ranking	Main Food Description	EPA + DHA density (mg/100g of food)
1	Roe, sturgeon	6548
2	Mackerel, baked/broiled	2351
3	Sardines, skinless, boneless, water-packed	2149
4	Herring, baked/broiled	2024
5	Squid, dried	1848
6	Mackerel, floured/breaded, fried	1607
7	Salmon, canned	1587
8	Sardines with tomato-based sauce (mixture)	1396
9	Herring, pickled	1389
10	Salmon, baked/broiled	1079
11	Salmon, cooked, cooking method NS	1056
12	Salmon, steamed/poached	1050
13	Trout, baked/broiled	1009
14	Sardines, canned in oil	983
15	Sardines, cooked	982
16	Mussels, steamed/poached	875
17	Brains, cooked	847
18	Salmon, floured/breaded, fried	834
19	Trout, breaded/battered, baked	765
20	Sea bass, steamed/poached	741

[1]Data are presented as mg/100g of food

Table 3.2B Top 20 foods with highest EPA + DHA density reportedly consumed by children ages 2-5, 6-11, and 12-18 years old[1]

Food Ranking	2-5 years old		6-11 years old		12-18 years old	
	Main food description	EPA + DHA density (mg/100g food)	Main food description	EPA + DHA density (mg/100g food)	Main food description	EPA + DHA density (mg/100g food)
1	Sardines, skinless, boneless, water-packed	2150	Squid, dried	1848	Roe, sturgeon	6548
2	Herring, pickled	1389	Salmon, canned	1587	Mackerel, baked/broiled	2351
3	Salmon, steamed/poached	1256	Salmon, baked/broiled	1079	Sardines, skinless, boneless, water-packed	2149
4	Salmon, baked/broiled	1088	Salmon, cooked, cooking method NS	1052	Herring, baked/broiled	2024
5	Trout, baked/broiled	1009	Salmon, floured/breaded, fried	987	Mackerel, floured/breaded, fried	1607
6	Sardines, cooked	982	Sardines, canned in oil	983	Salmon, canned	1587
7	Mussels, steamed/poached	875	Mussels, steamed/poached	875	Sardines with tomato-based sauce (mixture)	1396
8	Salmon, battered, fried	863	Salmon, battered, fried	863	Trout, baked/broiled	1083
9	Sea bass, steamed/poached	741	Trout, breaded/battered, baked	765	Salmon, baked/broiled	1077
10	Sea bass, baked/broiled	731	Sea bass, baked/broiled	742	Salmon, cooking method NS	1056
11	Trout, floured/breaded, fried	717	Pompano, baked/broiled	681	Sardines, cooked	983
12	Pompano, baked/broiled	715	Trout, floured/breaded, fried	665	Sardines, canned in oil	981
13	Swordfish, floured/breaded, fried	687	Trout, battered, fried	650	Salmon, steamed/poached	948
14	Trout, battered, fried	651	Salmon cake or patty	632	Mussels, steamed/poached	875
15	Salmon cake or patty	634	Fish, type NS, baked/broiled	608	Brains, cooked	847
16	Sea bass floured/breaded, fried	564	Sea bass, floured/breaded, fried	585	Salmon, floured/breaded, fried	834
17	Fish, type NS, baked/broiled	515	Fish, cooked, type and cooking method NS	584	Sea bass, baked/broiled	723
18	Fish, type NS, smoked	512	Squid, baked, broiled	583	Pompano, baked/broiled	702
19	Shrimp, cooked, cooking method NS	508	Oysters, raw	560	Trout, floured/breaded, fried	695
20	Fish, cooked, type and cooking method NS	505	Oysters, floured/breaded, fried	534	Oysters, steamed	695

[1]Data are presented as mg/100g of food

Table 3.3 Highest mean EPA+DHA intakes by foods reportedly consumed by children ages 2-18 years old[1]

Food Ranking	Main Food Description	Mean EPA+DHA intake (mg/d)	Number of children reporting food
1	Sardines, skinless, boneless, water-packed	2093	3
2	Salmon, cooked, NS as to cooking method	1724	3
3	Carp, floured or breaded, fried	1538	1
4	Fish, NS as to type, battered, fried	1224	16
5	Salmon, steamed or poached	1148	3
6	Salmon, baked or broiled	1124	34
7	Trout, breaded or battered, baked	1050	1
8	Shrimp creole, with rice	987	4
9	Salmon, floured or breaded, fried	935	2
10	Sardines with tomato-based sauce (mixture)	932	1
11	Haddock, floured or breaded, fried	903	1
12	Sea bass, baked or broiled	895	3
13	Oysters, canned	877	1
14	Mackerel, floured or breaded, fried	868	1
15	Sea bass, floured or breaded, fried	858	3
16	Mussels, steamed/poached	804	3
17	Squid, dried	776	1
18	Swordfish, floured/breaded, fried	731	2
19	Herring, pickled	690	3
20	Scallops, baked/broiled	675	1

[1]Data are presented as mean EPA and DHA intake in mg/day and number of children reportedly consuming each food

Table 3.4 The top 20 foods most children reportedly consume that contain EPA+DHA[1]

Food Ranking	Main Food Description	Number of children reporting food	EPA + DHA intake (mg/d)
1	Ice cream, regular, flavors other than chocolate	989	3.6 ± < 0.001
2	Salty snacks, corn or cornmeal base, tortilla chips	581	2.4 ± < 0.001
3	Egg omelet or scrambled egg, fat added in cooking	537	39.0 ± 0.002
4	Chicken or turkey loaf, prepackaged or deli, luncheon meat	501	7.4 ± 0.001
5	Pancakes, plain	409	1.2 ± < 0.001
6	Waffle, plain	395	12.1 ± 0.001
7	Chicken patty, fillet, or tenders, breaded, cooked	354	4.5 ± < 0.001
8	Egg, whole, fried	353	31.8 ± 0.001
9	Spaghetti with tomato sauce and meatballs, meat sauce, or meat sauce and meatballs	328	3.5 ± < 0.001
10	Cheerios	325	6.0 ± < 0.001
11	Froot Loops	297	1.7 ± < 0.001
12	Chicken, drumstick, coated, baked or fried, prepared with skin, skin/coating eaten	281	35.0 ± 0.002
13	Chicken, wing, coated, baked or fried, prepared with skin, skin/coating, eaten	275	45.6 ± 0.003
14	Bread, white	240	1.1 ± < 0.001
15	Egg omelet or scrambled egg, fat not added in cooking	234	42.0 ± 0.002
16	Roll, sweet, cinnamon bun, frosted	229	2.3 ± < 0.001
17	Chicken, NS as to part and cooking method, NS as to skin eaten	227	40.5 ± 0.003
18	Cheese, processed, American or Cheddar type	226	3.9 ± < 0.001
19	Corn dog (Frankfurter or hot dog with cornbread coating)	225	3.5 ± < 0.001
20	Egg omelet or scrambled egg, NS as to fat added in cooking	210	40.7 ± 0.003

[1]Data are presented as mean ± SE of EPA and DHA intake in mg/d, rank-ordered by number of children reportedly consuming foods that contain EPA and DHA

Table 3.5A Greatest contributing food groups (mean ± standard error) to dietary intake of EPA and DHA among US children ages 2-18 years old, NHANES 2003-2010[1]

Food Group	EPA+DHA intake (mg/d)	Number of children reporting food[2]
Fish and shellfish	373 ± 0.026	785
Meat, poultry, fish with nonmeat items	53 ± 0.004	2248
Frozen & shelf-stable plate meals, soups & gravies	47 ± 0.006	304
Egg mixtures	40 ± 0.001	1611
Poultry	32 ± 0.001	3156
Eggs	30 ± 0.001	625
Grain mixtures, frozen plate meals, soups	16 ± 0.001	2168
Pancakes, waffles, French toast, other grain products	8 ± < 0.001	1134
Organ meats, sausages and lunchmeats, and meat spreads	6 ± < 0.001	1116
White potatoes and Puerto Rican starchy vegetables	6 ± < 0.001	260
Other vegetables	5 ± < 0.001	85
Cereals, not cooked or NS as to cooked	5 ± < 0.001	1546
Quick breads	4 ± < 0.001	329
Salad dressings	4 ± 0.001	63
Beef	4 ± < 0.001	463
Cakes, cookies, pies, and pastries	4 ± < 0.001	2199
Cheeses	3 ± < 0.001	545
Milk desserts, sauces, gravies	3 ± < 0.001	1723
Pork	3 ± < 0.001	78
Crackers and salty snacks from grain products	3 ± < 0.001	598

[1]Data are presented as mean ± SE of EPA and DHA intake in mg/d, rank-ordered by EPA and DHA intake with n = number of children reportedly consuming food

[2]Food groups with less than 15 children reportedly consuming at least one food from the food group are not shown.

Table 3.5B Greatest contributing food groups (mean ± standard error) to dietary intake of EPA and DHA among US children ages 2-5, 6-11, and 12-18 years old, NHANES 2003-2010[1]

2-5 years old			6-11 years old			12-18 years old		
Food Group	EPA+DHA intake (mg/d)	Number of children reporting food[2]	Food Group	EPA+DHA intake (mg/d)	Number of children reporting food[2]	Food Group	EPA+DHA intake (mg/d)	Number of children reporting food[2]
Fish and shellfish	282 ± 0.020	206	Fish and shellfish	309 ± 0.026	242	Fish and shellfish	491 ± 0.055	337
Frozen & shelf-stable plate meals, soups & gravies	51 ± 0.006	117	Meat, poultry, fish with nonmeat items	48 ± 0.005	676	Meat, poultry, fish with nonmeat items	67 ± 0.007	1040
Egg mixtures	33 ± 0.002	521	Egg mixtures	41 ± 0.002	508	Frozen & shelf-stable plate meals, soups & gravies	59 ± 0.013	86
Meat, poultry, fish with nonmeat items	33 ± 0.004	532	Frozen & shelf-stable plate meals, soups & gravies	32 ± 0.006	101	Egg mixtures	44 ± 0.002	582
Eggs	24 ± 0.002	190	Eggs	30 ± 0.001	209	Poultry	40 ± 0.002	1327
Poultry	20 ± 0.001	822	Poultry	28 ± 0.001	1007	Eggs	34 ± 0.002	226
Grain mixtures, frozen plate meals, soups	11 ± 0.001	484	Grain mixtures, frozen plate meals, soups	14 ± 0.002	767	Grain mixtures, frozen plate meals, soups	19 ± 0.001	917
Other vegetables	11 ± 0.001	15	Pancakes, waffles, French toast, other grain products	8 ± 0.001	473	Pancakes, waffles, French toast, other grain products	11 ± 0.001	368
Pancakes, waffles, French toast, other grain products	6 ± < 0.001	293	White potatoes and Puerto Rican starchy vegetables	5 ± 0.001	106	Organ meats, sausages and lunchmeats, and meat spreads	7 ± 0.001	459

[1]Data are presented as mean ± SE of EPA and DHA intake in mg/d, rank-ordered by EPA and DHA intake with n = number of children reportedly consuming food.
[2]Food groups with less than 15 children reportedly consuming at least one food from the food group are not shown.

CHAPTER 4. DEVELOPMENT OF CHILD FRIENDLY FISH DISHES TO INCREASE YOUNG CHILDREN'S ACCEPTANCE AND CONSUMPTION OF FISH

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4.1. Abstract

Background: The Dietary Guidelines for Americans 2010 recommend that Americans ages two years and older consume seafood, especially fish high in n-3 polyunsaturated fatty acids, at least twice a week. Although fish is of particular importance during childhood to support proper brain and eye development, it is under-consumed in the US pediatric population. This study examined if substituting salmon for chicken would increase preschooler's fish consumption.

Methods: Two-to-five year old children (n=45) were served eight lunches (four pairs of comparable chicken versus salmon dishes) twice, totaling sixteen lunches over a period of three months to test the hypothesis that children will consume fish at least once a week, thus increasing docosahexaenoic acid (DHA) intake. The plate waste method was used to collect intake data and consumption of total energy and DHA intake in the chicken and the fish dishes were compared using contrasts within a mixed effect ANOVA (significance at $P<0.05$).

Results: Dietary intake estimates showed that there were no significant differences in energy intake when the chicken and fish dishes looked similar (macaroni and cheese and wraps), but when the fish dishes looked new (nuggets and dumplings), energy intake on fish days was lower than on the chicken day. DHA intake increased significantly on all days the fish was served.

Conclusions: This pilot study indicates that the Dietary Guidelines for Americans 2010 can be better followed if salmon is incorporated into familiar dishes such as salad wraps or macaroni and cheese, in the childcare setting, move toward meeting recommendations. Although fish is more expensive, childcare centers may serve this highly nutritious protein once a week without experiencing undue amounts of food wastes if incorporated into well-accepted main dishes. Further studies in larger and more diverse samples of children, different experimental dishes, and longer exposure periods may elucidate additional venues to increase children's diet quality by increasing consumption of oily fish.

Keywords: Fish consumption; Salmon; Diet quality; Young children; Acceptance; n-3 PUFAs; DHA

4.2. Introduction

The polyunsaturated fatty acid (PUFA) known to be critical for brain and eye development, docosahexaenoic acid (DHA, 22:6n-3), supports nervous tissue growth and function, such as learning and memory (24, 49). The parent 18-carbon fatty acid, α-linolenic acid (ALA), can be converted to various long-chain n-3 PUFAs, including eicosapentaenoic acid (EPA) and DHA. ALA is found in plant-based foods, with higher levels in soybean, canola, and flax seed oils. Another PUFA, the 18-carbon n-6 fatty acid linoleic acid (LA) is the precursor of n-6 fatty acids and is abundant in modern food supplies, as it contributes more than 50% of all the fatty acids in soybean, corn, safflower, and sunflower oils.

The conversion of ALA to DHA is very inefficient. Using compartment models, the conversion rate has been estimated to be less than 1% (15, 16). Also, measurement of peak or area-under-the-curve plasma contents of previously labeled fatty acids showed that less than 8% of ALA is converted into EPA and less than 4% to DHA in men (12, 114-116); in women, less than 21% of ALA is converted to EPA and less than 9% to DHA (13). Thus, consumption of dietary sources of DHA during the years of brain development (up to age 25) (29) is critical for children's cognitive functioning.

Once obtained from the diet, ALA can be metabolized to EPA, while LA is metabolized using the same enzymes to the 20-carbon chain n-6 fatty acid, arachidonic acid (23). The pathway generally accepted for metabolism of EPA to DHA involves elongation of the fatty acid carbon chain to yield DHA (117). In animals, an intake of 1% energy from LA, with an LA to ALA ratio of 2:1 or lower, supports high levels of DHA in the developing brain (52, 118). However, high intakes of LA inhibit desaturation of ALA to EPA and DHA and reduce accretion of DHA in the brain, retina, and other organs (52, 118-121). This means that dietary n-3 PUFAs, the amount and type of n-3 PUFA (ALA and DHA), and high LA determine DHA accretion in the developing brain and retina. When n-3 PUFA intake is inadequate, DHA decreases and n-6 PUFAs increase in the brain. Human milk and infant formulas now provide more than 3% energy from LA, which suggests circulating levels and tissue levels of DHA will be low unless DHA is provided (49).

DHA is only found in animal tissue lipids, with oily fish being the best dietary source. It is not present in plant sources (such as vegetable fats and oils, grains, nuts, and seeds), although those may provide other n-3 PUFAs (49). Because humans lack specific desaturases, they are unable to form n-3 or n-6 PUFAs de novo and must obtain these PUFAs from their diet (23). DHA is the major n-3 PUFA esterified in the glycerophospholipids that form the structural matrix of brain grey matter and retinal membranes (50, 51). Therefore, DHA accumulation in the brain and retina as well as in other organs depends on the amount and types of n-3 PUFAs consumed in the diet. In addition, dietary intake of n-6 PUFAs plays a role, as n-6 PUFA interact and compete with n-3 PUFAs in the fatty acid metabolic pathway (5-8, 52-54).

The in vivo conversion of ALA to DHA is not very efficient, and the Dietary Guidelines for Americans 2010 recommend consuming more foods rich in EPA and DHA by eating seafood at least twice a week. Health-conscious parents strive to offer food sources of DHA to incorporate into their children’s diets but in most US regions fish consumption, especially intake of oily fish, are very low and children may not accept fish into their diet easily.

Prenatal DHA availability is determined by maternal dietary DHA intake during pregnancy and has been shown to have a significant effect on quality of movement in seven-year-old children (36). Prenatal DHA availability is critical for brain development in utero but also later in life. For that reason, DHA supplementation in infant formula is recommended as it has been shown to lead to visual acuity and IQ maturation similar to that of breast-fed infants (122). Even past the usual age of weaning, the brain is not fully developed. The frontal lobes of the brain, which are responsible for executive functions, develop in spurts between birth-to-two years of age, from seven-to-nine years of age, and in the mid-teenage years – up to the age of 25 years old (29). Hence, adequate intake of DHA prior to adulthood is imperative to support healthy brain development and consumption of DHA-rich foods, such as oily fish, is recommended for all Americans.

According to the Institute of Medicine report (72), it is recommended that 2-5 year old children consume two age-appropriate servings (1-to 2-ounce) of seafood per week. However, previous research has shown that fish consumption is less than adequate, especially when compared to pasta, a dish that is common in the Western diet. In a previous study (76), when pasta was served to 23 two-to-five year old children, intake was 81% greater than when fish was served. This is the case even for immigrants from Asia who would normally have a high fish intake (77), but have been submersed into Western culture. When assessing the dietary patterns of Korean adolescents, fish dishes such as kimchi, fish cake soup, and fish cutlets comprised the majority of meals, whereas Korean-American adolescents consumed a more typical American diet of milk, soda, hamburgers, etc. (77).

Despite the benefits of DHA in the diet, Western diets are low in n-3 PUFAs, especially ALA found in plant oils and DHA found in fish (49). According to the Institute of Medicine and National Academy of Sciences (72), the median intake of EPA and DHA in adults is 0.05% of dietary energy. Contrary to popular belief, even an individual meeting the estimated intake recommendations for n-3 PUFA intake may consume less than optimal amounts of DHA, if the majority of the n-3 PUFAs are from plants. Consequently, this becomes an issue for the developing brain. Western diets low in n-3 PUFAs and high in n-6 PUFAs contribute to poor brain development and function (49). By adding oily fish to children's diets, DHA intake would increase, and aid in the prevention of poor brain development. Dalton et al. (29) showed that when seven-to-nine year old children's diets were supplemented with a fish-flour spread rich in n-3 PUFAs, verbal learning ability and memory were improved. Thus, effective ways to encourage habitual consumption of a diet high in DHA are warranted.

For children, the general requirements of total fat and fatty acids have not yet been adequately established (69). Currently, recommended DHA intake levels are based on values calculated on a per kilogram (kg) body weight basis. However, according to Koletzko et al. (70), EPA and DHA intake recommendations using the estimation based on body weight may result in suboptimal DHA amounts in 2-12 year old children because their DHA needs are higher relative to their body weight. In adulthood, DHA needs are lower compared to the time from birth to age 25.

Once the importance of EPA and DHA was established, numerous alternative sources of n-3 PUFAs were developed. The only natural sources of DHA are marine food sources, such as fish and seaweed, and their products, such as fish and algal oil.

However, food companies have taken the initiative to incorporate DHA and other n-3 PUFAs into foods such as breads, pastas, milk, eggs, processed meats, salad dressings, margarines, mayonnaise, peanut butters, pizzas, nutrition bars, cereals, yogurts, and juices (58). Although the fortification with DHA for these processed foods is based on marine sources, such as fish or algal oils, there are many issues that should be considered. The bioavailability of the n-3 PUFAs in synthetic form is understudied. Furthermore, foods with added DHA usually only contain 12-50 mg EPA and DHA combined. The mean DHA amount consumed per one serving of these foods is 32 mg, which is equivalent to less than a teaspoon of salmon. With product claims such as "good source of DHA," parents may become misled and confused. While the population seems to have accepted these synthetic sources of DHA and other n-3 PUFAs, skepticism remains in the field of nutrition.

The Dietary Guidelines for Americans 2010 recommend consuming seafood at least twice a week. Based on the estimated intake of DHA in Americans, especially American children, the development of venues to increase fish consumption by offering child-friendly fish dishes is a critical public health concern. The present pilot study was designed to explore two modes of offering oily fish to 2-5 year old children – by incorporating it in well-accepted dishes and by offering novel dishes. The specific aims of this research were to 1) substitute salmon for chicken without significantly affecting 2-5 year old children's energy intake and 2) increase children's intake of DHA by offering salmon for lunch. We hypothesized that offering canned or cooked salmon to preschoolers during lunch at the childcare center would lead to children's consumption of fish at least once a week.

4.3 Methods

4.3.1 Study Participants

Recruitment of participants was based upon attendance at the Ben and Maxine Miller Child Development Laboratory School, a childcare center located at Purdue University (West Lafayette, Indiana). As the unit of analysis was group-level mean consumption, no individual child was identified and all data were recorded and analyzed for the group of participating children. Eligibility was restricted to children between the ages of 2-5 years; exclusion criteria included the presence of food restrictions, food allergies, or digestive diseases, such as Crohn's Disease or Cystic Fibrosis. Forty-five children from three different classrooms participated in the study. This study was approved by the Institutional Review Board of Purdue University. As no individual data was collected, consent procedures consisted of approval by the director and teachers of the childcare in addition to verbal assent by each participating child prior to collecting data on each study day. Children's refusal to participate in the study, such as not wanting to rate the foods, was honored.

4.3.2 Study Design

This study functioned as a pilot study with one within-subject factor (meal). Over a period of three months, children were twice served the four regularly scheduled chicken main dishes and the study foods: salmon nuggets, salmon dumplings, salmon salad wraps, and salmon macaroni and cheese. The chicken main dishes were regularly scheduled once a week, and each main dish was served once a month. The salmon main dishes substituted for regularly scheduled items over the duration of the study, with a salmon main dish served once a week and each salmon main dish served once a month. During each study lunch, children rated the liking of the dish (appearance, taste, texture, smell, and overall liking). To calculate food intake and total energy consumption (in kilocalories), the plate waste method was employed. The plate waste method and scale to rate liking of foods are described below.

4.3.3 Dietary Assessment Methods

Children's liking of the chicken or fish dish was measured using a three-point Likert-type scale using smiley faces. First, the main dish was presented to the child on his or her plate with the rest of the lunch items and the child was asked to take a bite to taste the dish and then categorize the food as 'yummy,' 'yucky' or 'just okay.' The children were asked to provide five ratings for each main dish in regards to the appearance, taste, texture, smell and the overall liking of each main dish. The children's responses were recorded by the researcher and entered into excel sheets for analysis.

The plate-waste method was used to measure children's consumption of lunch and to determine percent waste, macronutrient consumption, and actual weight and caloric density of foods consumed and discarded. In each classroom, each food served at lunch was weighed in their respective serving bowls prior to being distributed to the children's lunch plates. The weight (in grams) of each food was recorded. Once lunchtime was over, the waste of each food was collected, combined with the respective leftover food, and weighed to determine how much of that particular food was not consumed. From there, the waste was subtracted from the initial weight to determine how much food the children consumed in grams to calculate energy consumption in kilocalories.

4.3.4 Experimental Meals

The four novel fish main dishes were designed to be similar to the regularly served chicken main dishes, which were already incorporated into the childcare center's 8-week menu rotation. The portion sizes of the meals were based on the United States Department of Agriculture (USDA) Food and Nutrition Service Child and Adult Care Food Program (CACFP) Child Meal Pattern for Lunch for 1-2 year olds and 3-5 year olds (123). The comparison of total energy (kcal/100g) and DHA (mg/100g) content provided by each main dish is reflected in Table 4.1. The fish-based dishes were designed to be of equivalent energy density as the chicken-based dishes.

The chicken-based dishes and the comparable fish-based dishes were a chicken patty versus salmon nuggets, chicken macaroni and cheese versus salmon macaroni and cheese, chicken salad wrap versus salmon salad wrap, and chicken stir-fry versus salmon dumplings. Due to the texture of salmon, a fish stir-fry would not have been acceptable to most children, thus, pot-sticker dumplings were served instead. Although canned pink salmon was directly substituted for canned chicken in the salad wrap and macaroni and cheese recipes, the other recipes required further development. To develop the salmon nuggets and salmon dumplings, cooked Atlantic salmon was pureed with canned Great Northern Beans to help provide a soft and smooth textured protein base for the salmon nuggets and dumplings. The salmon-bean mixture was prepared in two different ways: 1) lightly coated in breadcrumbs and baked to form salmon nuggets and 2) portioned into Wonton wrappers and steamed to form salmon dumplings. The four experimental dishes were developed and taste-tested in a preschool-age population. Based on the taste-test responses, the recipes were modified until at least 80% of the children liked the test foods.

4.3.5 Procedures

On each study day, teachers in participating classrooms were instructed to follow standard mealtime procedures for lunch. In each classroom the children would sit at a table together and were served lunch by a research assistant. Children were not encouraged to eat more or less than usual and were instructed not to share food. All food liking results and plate-waste measurements were entered into excel sheets for additional analysis. Children's intake was entered into the Nutrition Data System for Research (NDSR) 2012. Food intake was recorded as grams of food consumed and total energy for each food component at lunch and for the whole meal (kcal) was calculated as well as intake of DHA (mg). For a more accurate analysis of how much DHA was provided by the two types of salmon used in the study (canned and cooked), gas chromatography with a flame ionization detector was used.

The brand of canned salmon (3 lots) and cooked Atlantic salmon (1 fillet) were purchased from local stores around Lafayette, Indiana in 2013. From each lot, the total contents were combined and ground in a food processor to obtain a composite sample. The following methods have been replicated from a previous study by Shim, Dorworth, Lasrado, and Santerre (124). For determination of total fat, two composite samples were randomly chosen from each lot, thawed, and mixed well. A modified Folch method (125) was used to determine total fat concentration. Five grams of composite tissue was mixed with 100 mL of chloroform/methanol (2:1, v/v, HPLC grade for chloroform, pesticide grade for methanol, Fisher Scientific, Fair Lawn, N.J., U.S.A) for 2 h to extract the fat. The mixture was filtered (Whatman filter paper nr 1, 150-mm dia, Whatman Intl. Ltd. Maidstone, England) and 50 mL of 0.88% potassium chloride (ACS reagent, Sigma, St. Louis, Mo., U.S.A.) was added to the filtrate. After removing the aqueous layer (upper), the solvent (lower) was reduced by evaporation using a Turbo Vap® (Zymark Corp., Hopkinton, Mass, U.S.A.). The extract was transferred to a pre-weighed flask and placed in a desiccator overnight. Duplicated blanks were included in each run during the fat extraction. Ninety-five percent recovery of total fat was determined using a Standard Reference Material (SRM) (Lake Superior fish tissue 1946, Natl. Inst. of Standards and Technology, Gaithersburg, Md., U.S.A.). Determination of fatty acids was carried out using the AOAC method 991.39 (AOAC 2000). PUFAs, including LA, ALA, stearidonic acid (SDA), AA, EPA, docosapentaenoic acid (DPA), and DHA were quantified by gas chromatography with a flame ionization detector (GC/FID, Varian 3900 GC, CP-8400 auto sampler, CP-8410 auto injector, Varian Analytical Instruments, Walnut Creek, Calif., U.S.A.). Operating conditions were as follows: injection port temperature, 240 °C; detector temperature, 300 °C; oven programmed from 175 °C for 4 min to final hold temperature of 240 °C for 5 min with an increase of 3 °C/min; helium carrier gas (99.999% pure, Inweld, Inc., Lafayette, Ind., U.S.A.); and wall coated open tubular (WCOT) fused silica capillary column, 30 m x 0.32 mm, coated with Chrompack (CP) wax 52CB, DF 0.25 mm (CP 8843, Varian).

4.3.6 Statistical Analysis

All statistical analyses were conducted using the Statistical Analysis Software (version 9.3, 2010, SAS Institute Inc., Cary, NC). For the three-point Likert scale ratings, participants' responses were recorded and coded: '-1' for 'yucky,' '0' for 'okay,' and '+1' for 'yummy.' The values were entered for each participant, for each main dish (chicken and salmon), and for each category (appearance, taste, texture, smell, and overall liking). Mean responses were calculated. A two independent sample t-test was conducted to determine statistical differences between each of the food categories. Kilocalorie consumption per child and DHA consumption per child were analyzed using a mixed model analysis of variance. Factors included in the model were classroom (3 levels), main dish type (4 levels), food type (chicken and salmon), and the interaction between food type and main dish type. Contrasts were then used to compare food types for each main dish. Statistical significance was defined as $P<0.05$.

4.4 Results

Complete intake data were obtained for 45 children. Data were aggregated by classroom, and a total of 48 eating occasions (3 classrooms x 16 meals) were collected. The analysis of variance indicated that the interaction between main dish and food type was statistically significant ($P<0.0001$). To investigate this, contrasts were used to compare food types for each main dish. Means and standard deviations of energy and DHA intake of the main dishes at lunchtime are provided in Table 4.2. Energy intake decreased by 83% for salmon nuggets compared to the intake of the chicken patty (43 versus 256 kcal, $P<0.0001$). However, DHA intake increased by 550% (117 versus 18 mg, P=0.0024). Energy intake decreased by 54% for the salmon dumplings compared to the chicken stir-fry (50 versus 108 kcal, P=0.0120) but DHA intake increased by 722% (148 versus 18 mg, P=0.0001). No significant difference for energy intake was observed for the substitution of chicken in the wrap as energy intake decreased by 28% when the salmon salad wrap was served instead of the chicken salad wrap (78 versus 108 kcal, P=0.1916) but DHA intake increased by 15400% (155 versus 1 mg, P<0.0001). Likewise energy intake only decreased by 3% when the main course was salmon macaroni and cheese as compared to chicken macaroni and cheese (152 versus 148 kcal, P=0.8640) and DHA intake increased by 33800% (339 versus 1 mg, P<0.0001). Thus, in two of the substitutions (salmon dumplings and salmon nuggets), total energy intake decreased significantly, but DHA intake increased despite the reduction in total food intake. As for the salmon macaroni and cheese and salmon salad wraps, total energy intake was not significantly different, DHA intake increased, and these foods were so widely accepted by the children that the foods were permanently incorporated into the lunch menu of the childcare center.

The results of the Likert scale ratings used to determine liking of the main dishes are provided in Table 4.3. No significant differences were observed between the chicken and salmon macaroni and cheeses, the chicken and salmon salad wraps, or the chicken patty and salmon nuggets. As for the difference between the chicken stir-fry and salmon dumplings, only overall liking of the dumplings was significantly lower (P=0.001). Overall, the Likert scale ratings corresponded to energy consumed by participants. There were no significant differences in the ratings of the chicken and salmon macaroni and cheeses or the chicken and salmon salad wraps, and both of these pairs of main dishes did not have significant differences in energy intake. Conversely, there was a significant difference in overall liking and energy intake for the chicken stir-fry in comparison to the salmon dumplings.

4.5 Discussion

DHA supports healthy brain and eye development and oily fish are the best dietary sources of DHA. Therefore, changing children's consumption patterns to include oily fish is a critical public health issue. This study was designed to explore the feasibility of increasing preschooler's fish consumption to help meet the dietary guideline for seafood consumption and to increase DHA intake. Results showed that although children's intake of the main dish decreased when some of the salmon-based foods were served, DHA intake was significantly higher than when the regularly scheduled chicken-based dishes were served.

In the two instances where the salmon main dishes consisted of a mixture of cooked beans and Atlantic salmon and did not resemble the chicken main dishes in appearance (and were unfamiliar to the children), main dish intake significantly decreased (salmon nuggets and salmon dumplings) (Table 4.2). This finding was expected as it is well documented that young children are resistant to accepting new foods into the diet and consumption of novel foods only increases with repeated exposure. In this particular study, only two exposures were provided and it is probable that consumption would have increased if the children had increased exposures to the two novel fish dishes.

However, in the two instances where the salmon main dishes incorporated canned pink salmon and did resemble the chicken main dishes in appearance (and were therefore familiar to the children), main dish intake did not significantly decrease (salmon salad wrap and salmon macaroni and cheese) (Table 4.2). In addition, there were no significant differences between the children's ratings on appearance, taste, texture, smell, and overall liking of the chicken and salmon versions of the salad wraps and macaroni and cheese main dishes (Table 4.3). Since oily fish is the best dietary source of DHA (49), our results indicate that modifying main dishes to incorporate salmon can prove to be an effective approach to increase DHA intake at meals.

With the exception of the overall liking of the dumplings, children did not report any difference in the liking of the five characteristics of the four different salmon main dishes versus the chicken main dishes studied (Table 4.3). Therefore, the results of this study support our hypothesis that 2-5 year old children will consume fish at least once a week and therefore increase DHA intake. It must be noted that these four fish dishes were created to be similar to the regularly served chicken main dishes. By children rating the salmon and chicken dishes similarly on the itemized list of food characteristics, this study demonstrates that it is possible to improve young children's diet quality by serving fish in childcare centers.

Study results support the premise that parents and caretakers of children should introduce children to fish at a young age. Through repeated exposure to certain foods, children develop a liking for the food's characteristics (appearance, smell, taste, and texture) (126). If oily fish were offered to children as a protein source at least twice a week, children would predictably choose to eat more fish, thereby increasing their DHA intake. Although the main dishes tested in this study were only representative of a small portion of the main dishes children usually consume, providing fish at least once a week can have an additive effect on children's mean fish consumption and DHA intake. Whether fish is served on its own (such as a salmon filet) or incorporated into mixed dishes (such as salmon macaroni and cheese or a salmon salad wrap), children's mean daily intake of DHA will increase.

The present study had several strengths and limitations. This study was highly innovative as we are not aware of any other studies on the development of child-friendly fish dishes in an effort to increase children's oily fish consumption. Due to the results of this study, especially the high acceptance of the salmon macaroni and cheese and the salmon salad wrap, the childcare menu was revised to incorporate these two dishes into the childcare center's 8-week menu rotation. Since data were collected on the group level, individual changes in intake or liking of the foods were not identified. The study was based on a university population (higher parent education, more international diversity, less domestic ethnic diversity, etc.) and was therefore not representative of the US pediatric population. The results of this study indicate that children's intake in a childcare setting can be modified to improve overall diet quality and support children's growth and development.

Future research on this topic should be based on larger and more diverse samples of children and include individual data, provide more exposures, and a larger variety of fish-based foods. Despite the limitations of this study, the findings strongly indicate that it is feasible and advisable for childcare centers to include offering high-fat fish once a week. Although some children may not consume as much of the fish-based lunch initially, this change in the menu may help children adopt a healthier diet for life as well as provide essential n-3 PUFAs, specifically DHA, which are critical in the development of the nervous system.

Table 4.1 Energy density of each main entrée (kcal/100g) and DHA provided (mg/100g)

Main entrée	Energy density (kcal/100g)	DHA (mg/100g)
Chicken Stir-fry, fresh boneless chicken thighs, stir-fried	236.3	38.8
Chicken Macaroni & Cheese, fully cooked, diced, dark and white blend	205.0	1.5
Chicken Breast Patty, breaded, baked	263.2	18.4
Chicken Salad Wrap, fully cooked, diced, dark and white blend	280.4	3.6
Salmon Dumplings, cooked Atlantic salmon pureed with Great Northern Beans, steamed	197.1	584.5
Salmon Macaroni & Cheese, canned pink salmon	198.3	453.9
Salmon Nuggets, cooked Atlantic salmon pureed with Great Northern Beans, baked	215.4	584.5
Salmon Salad Wrap, canned pink salmon	229.5	453.9

Table 4.2 Comparison of children's energy and DHA intake of main entrée (chicken versus salmon) (mean ± SD)

Meal	Main entrée energy (kcal)	P-value	DHA (mg)	P-value
Chicken Patty	256 ± 40	<0.0001	18 ± 3	0.0024
Salmon Nuggets	43 ± 30		117 ± 83	
Chicken Macaroni & Cheese	152 ± 58	0.8640	1 ± 0	<0.0001
Salmon Macaroni & Cheese	148 ± 50		339 ± 115	
Chicken Stir Fry	108 ± 26	0.0120	18 ± 4	<0.0001
Salmon Dumplings	50 ± 16		148 ± 46	
Chicken Salad Wrap	108 ± 40	0.1916	1 ± 1	0.0001
Salmon Salad Wrap	78 ± 32		155 ± 62	

Table 4.3 Differences between chicken entrée scores and salmon entrée scores[1]

	Appearance	Taste	Texture	Smell	Overall
			Difference (p-value)		
Chicken Patty versus Salmon Nuggets	0.187 (0.145)	0.310 (0.006)	0.2711 (0.038)	0.169 (0.180)	0.291 (0.015)
Chicken versus Salmon Macaroni and Cheese	0.056 (0.671)	0.108 (0.291)	0.174 (0.162)	0.115 (0.331)	0.100 (0.345)
Chicken Stir-Fry versus Salmon Dumplings	0.290 (0.051)	0.210 (0.128)	0.189 (0.141)	0.193 (0.196)	0.446 (0.001)*
Chicken versus Salmon Salad Wrap	0.055 (0.708)	0.024 (0.865)	0.220 (0.115)	0.204 (0.155)	0.144 (0.305)

[1]Based on the nature of the coding (-1 for dislike, 0 for indifferent, or +1 for like), the difference scores ranged from "-2" to "+2." A value of zero indicated the children did not prefer one version of the food over the other, and the one-sample t-test tested whether or not the difference was equal to zero.
*Statistical significance at P<0.0025 when using the Bonferroni adjustment.

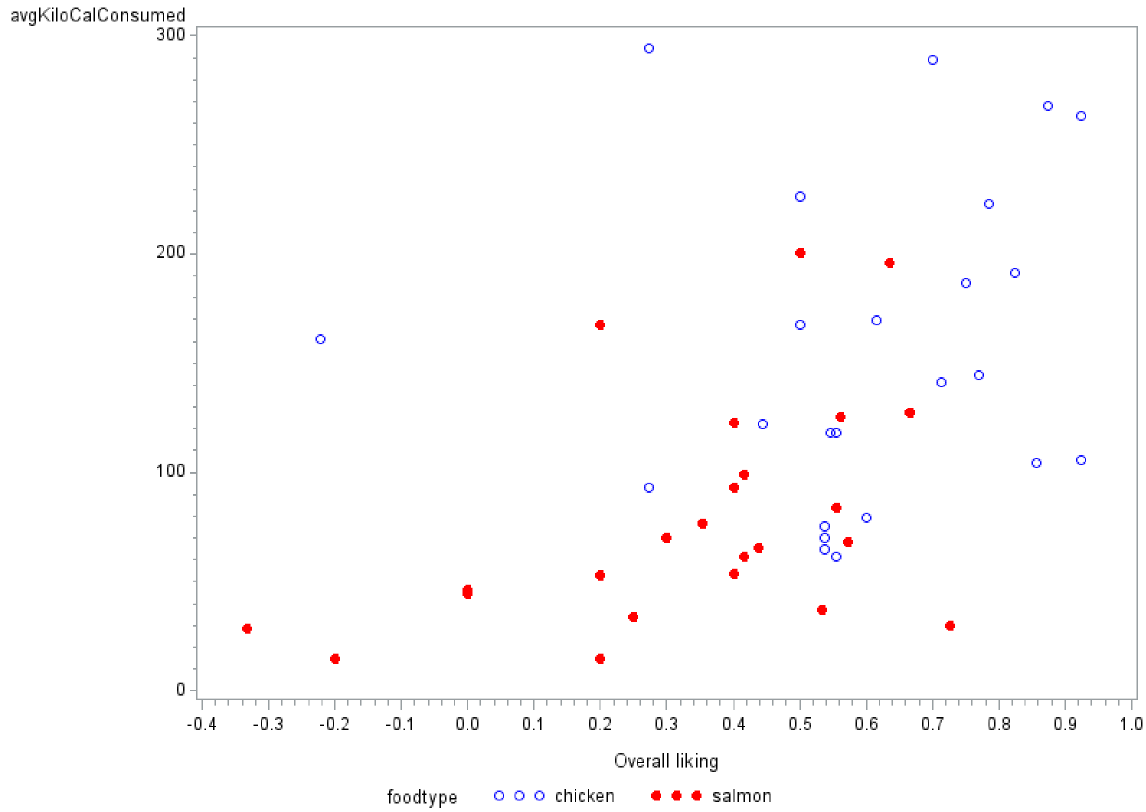

Figure 1 Correlation between mean energy intake per meal (y-axis) and overall liking (x-axis) of each study lunch, p = 0.0015[1]

[1]Each dot represents one food in one classroom during one visit e.g. salmon salad wrap in classroom 1 on the first Thursday of the month. Per one unit increase in liking rating (x-axis), mean energy intake per meal increased by 114 kcal and was statistically significant with a p-value = 0.0015. As demonstrated visually, as overall liking increased, mean energy intake increased.

CHAPTER 5. CONCLUSIONS

5.1 Conclusions

The results of these studies indicate that 2-18 year old children's actual intake of EPA and DHA are not meeting current target intakes set forth by the WHO and FAO. Instead of consuming food sources high in EPA and DHA, the majority of children are consuming larger quantities of foods that have minimal EPA and DHA content. However, we have shown that by incorporating oily fish (such as salmon – the best dietary source of DHA) (49) into child-friendly lunches, we can increase fish consumption and significantly increase DHA intake in 2-5 year old children. Two-to-five year olds were the target age group, as this is the period during development when food preferences are established (127, 128).

EPA and DHA play important roles throughout the human lifespan, but especially during childhood due to rapid growth and development. EPA provides many benefits toward cardiovascular health whereas DHA is more important for optimal cell membrane function and is vital to the development of the brain and retina (129). Due to the importance of EPA and DHA for growth, development, and health, it is essential to consume foods that contain these conditionally essential fatty acids.

An important factor that determines children's consumption of fish and other foods high in EPA and DHA is availability. Availability is determined by the caretaker's (parent or caregiver's) access and finances. Once the food is available, care takers can improve consumption if they create a social environment conducive to eating fish i.e. food presentation, food exposure, and being a role model by eating the same food with the children (130). There are many factors affecting children's acceptance of a food. These include biological (hunger, appetite, taste), economic (cost, family income), physical (access, education, skills, time), social (class, culture, social context), and psychological (personality, exposure, motivation, emotions) factors (131, 132).

The NHANES analysis provided a population-based assessment of fish and shellfish consumption and EPA and DHA intake in 2-18 year old children. Data from this study describe the foods reportedly consumed by children that have the highest EPA and DHA density, the foods that are consumed the most by children (by quantity of food and number of children), and the food groups that have the greatest contribution to dietary intake of EPA and DHA. In addition, findings clearly indicate the gap between current EPA and DHA intake levels in comparison to the guidelines set forth by the WHO and FAO.

As the results of the nutrition intervention showed, introducing oily fish, such as salmon, into childcare center's lunch menus, increased children's exposure and consumption to oily fish. By increasing children's fish acceptance at a young age, their preference for fish will likely remain consistent and EPA and will remain higher. Overall, the above research provides evidence for the importance of further developing ways to increase children's consumption of food with high EPA and DHA density to support healthy growth and development of the brain and retina.

5.2 Implications for Future Research

Future research should include studies directly measuring children's DHA intake on the individual level, using larger and more diverse populations, in different settings (childcare setting versus home setting), and differing taste exposures (fish nuggets, dumplings, wraps, and macaroni and cheese). In addition, the inclusion of a larger variety of lunches incorporating oily fish into the meals would help to determine children's acceptance and consumption patterns of fish-based meals. The results of the nationally representative analysis showed the importance of monitoring dietary intake behavior changes. Future studies should also take into consideration the demographics of specific populations i.e. socioeconomic status, cultural norms, access to seafood dependent on geographical location, et cetera. Additional barriers should be investigated such as beliefs about fish consumption, availability, and preparation ability by parents, parental eating habits, and cost. For example, women with children five years old and younger and with a PIR < 1.85 are eligible for WIC benefits. Currently, WIC food packages for fully breastfeeding mothers include compensation for canned pink salmon and canned white tuna. However, only children that are being fully breastfed or children that have younger siblings who are fully breastfed benefit from this WIC food package. Revising the food package to allow all WIC-eligible children to purchase canned fish will help support the public health effort to increase children's fish consumption.

5.3 Policy Implications

The present studies provide novel information about the current status of children's fish and shellfish consumption, the food sources that provide the most EPA and DHA to children, and the food sources most children are reportedly consuming that contain any EPA and DHA. Current EPA and DHA intake by children is suboptimal, but there are plausible approaches to increase their intake by promoting fish consumption in childcare and school settings. The low intakes of EPA and DHA demonstrate the need to develop a course of action to increase EPA and DHA intake. We have demonstrated the practicality of incorporating child-friendly salmon dishes into the lunch menu of a childcare center. The combination of nationally representative data with a feasibility study to increase EPA and DHA intake provide a gateway for implementing initiatives to improve the diet quality of children. Results from this work are applicable to schools, communities, families, and individuals that aim to improve the dietary behaviors of American children.

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APPENDICES

Appendix A Eight-week rotation menu of local childcare center during duration of community-based, feasibility study

Week 1 Menu Rotation

	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	Chicken patty on whole wheat bun	Teriyaki chicken w/rice	Turkey meatloaf w/biscuits	Salmon Macaroni & Cheese	Cheese Ravioli
Veggie	Salad w/tomatoes	Steamed veggies	Carrots	Zucchini	Green beans
Fruit	Apples	Peaches	Grapes	Orange wedges	Pears
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Week 2 Menu Rotation

DAY	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	Sloppy Joe on whole wheat bun	Chicken enchiladas	Pancakes and turkey sausage	Salmon Nuggets	Chicken Salad Wrap
Veggie	Steamed peas	Green beans	Tater tots	Broccoli & Cauliflower	Carrots
Fruit	Watermelon	Grapes	Oranges	Apples	Pineapple
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Week 3 Menu Rotation

DAY	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	Chicken Pot Pie	Turkey Tacos	Chicken Macaroni & Cheese	Salmon Dumplings w/wild rice	Spaghetti and Meatballs
Veggie	Carrots	Lettuce/tomatoes/ olives	Tomato/cucumber	Cauliflower blend	Zucchini/squash
Fruit	Fresh Fruit	Fresh Fruit	Grapes	Fresh Fruit	Mandarin oranges
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Week 4 Menu Rotation

DAY	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	4x6 Tony's pizza	Chicken stir-fry w/rice	Turkey Sandwiches	Salmon salad wrap	Toasted cheese sandwich
Veggie	Salad w/carrot and cucumber	Stir-fry veggies	Peas & corn	Sweet potato wedges	Cherry tomatoes
Fruit	Diced pears	Fresh fruit	Fresh fruit	Blueberries	Canned fruit
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Week 5 Menu Rotation

DAY	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	Chicken and noodles w/dinner roll	Pasta Primavera	Turkey Meatloaf w/biscuits	Salmon Macaroni & Cheese	Cheese pizza
Veggie	Diced carrots	Italian blend vegetables	Carrots	Steamed veggies	Peas
Fruit	Apple wedges	Fresh fruit	Grapes	Fresh fruit	Blueberries
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Week 6 Menu Rotation

DAY	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	Grilled chicken w/breadstick	Salmon Salad Wrap	Turkey Sausage & Pancakes	Fish Nuggets w/wheat roll	Tofu & Rice
Veggie	Salad	Salad	Cucumbers	Green beans	Stir-fry veggies
Fruit	Watermelon	Strawberries	Oranges	Fresh fruits	Pineapple
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Week 7 Menu Rotation

DAY	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	Beef & Vegetable Stroganoff	Salmon Dumplings w/white rice	Chicken Macaroni & Cheese	Pork loin w/wheat roll	Chicken Fajitas
Veggie	Stroganoff vegetables	Salad	Cucumbers	Mashed potatoes	Zucchini & squash
Fruit	Strawberries	Fresh fruit	Grapes	Orange wedges	Watermelon
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Week 8 Menu Rotation

DAY	Monday	Tuesday	Wednesday	Thursday	Friday
Entrée	Chicken pot pie	Ham & Cheese Sandwich	Turkey w/whole wheat bread	Salmon nuggets w/wheat roll	Toasted cheese sandwich
Veggie	Peas & carrots	Mixed green salad	Corn	Spinach & corn	Tomato soup
Fruit	Diced pineapple	Fresh fruit	Fresh fruit	Apple wedges	Fresh fruit
Dairy	Milk	Milk	Milk	Milk	Milk
Dessert					Cookie

Appendix B Data from gas chromatography with flame ionization detector to quantify the polyunsaturated fatty acids in the cooked and canned salmon incorporated in the child-friendly salmon dishes

		Canned pink salmon	Cooked, farmed, Atlantic salmon
		mg/100g tissue	
SFA	12:0	1 ± 0	4.8 ± 0.9
	14:0	75.8 ± 2	345.5 ± 74
	16:0	313.4 ± 9.7	1344.1 ± 288.9
	18:0	63.9 ± 2.1	338.3 ± 97.4
	24:0	3.3 ± 0.1	11.8 ± 2.1
	ΣSFA	457	2045
MUFA	14:1n5	3.7 ± 0.1	9.8 ± 2.1
	16:1n7	71.6 ± 2	367.6 ± 79.3
	18:1n7	43 ± 1.6	427.3 ± 122.8
	18:1n9	190.5 ± 6.7	5660.7 ± 1617.4
	20:1n9	61.5 ± 2.1	471.6 ± 100.2
	22:1n9	24.3 ± 0.8	69.4 ± 15.1
	ΣMUFA	395	7006
PUFA	16:2n4	3.7 ± 0.1	29.7 ± 5.7
	16:3n4	1.8 ± 0.2	22 ± 4.7
	18:2n6 (LA)	26.1 ± 1.4	1883.7 ± 538.5
	18:3n3 (ALA)	21.2 ± 0.9	767.9 ± 220.7
	18:3n4	2.9 ± 0.3	20 ± 3.7
	18:3n6	10.9 ± 0.3	13.5 ± 2.9
	18:4n3 (SDA)	48.2 ± 1.5	88.3 ± 19
	20:4n3	29.3 ± 1.1	97.1 ± 20.6
	20:4n6 (ARA)	12.6 ± 0.5	39.3 ± 8.2
	20:5n3 (EPA)	185 ± 7.4	431 ± 91.3
	22:5n3 (DPA)	61.9 ± 2.9	174.2 ± 36.8
	22:6n3 (DHA)	453.7 ± 20.5	584.3 ± 120.2
	Σ(n-3) FA	799	2143
	Σ(n-6) FA	50	1936
	ΣPUFA	857	4151

VITA

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Lyndsey Rae Herdzina-Huss was born in Fort Wayne, Indiana and raised in Ossian, Indiana. She received a BS in the Coordinated Program in Dietetics and a Minor in Child Development and Family Studies from Purdue University of West Lafayette, Indiana in May 2012. As part of the Coordinated Program in Dietetics, she completed a dietetic internship to become a Registered Dietitian. In the fall of 2012, she entered the Interdepartmental Nutrition Program for graduate students at Purdue University under the direction and mentorship of Dr. Sibylle Kranz with a research focus on community nutrition in the pediatric population. Throughout her MS, she has been a Purdue University Candidate for the International Life Science Institute (ILSI) Graduate Student Summit and a Purdue University Graduate Fellow.